EXPERIMENTS ON THE EFFECTS OF INJECTION OF EGG-ALBUMEN AND SOME OTHER PROTEIDS.

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INTRODUCTION.

Although the occurrence of albuminuria after injection of certain proteids, notably egg-albumen, has long been recognized, many details of this phenomenon have received but scant attention. At the time when this research was started, in the spring of 1897, very little was known concerning the ratio of the proteid which is excreted, the time required for excretion, and the factors which influence these; nor were there any very definite data about the fate of that portion which is retained, about the changes occurring in the kidneys, and about certain toxic effects produced. Since then a number of important papers dealing with this subject have made their appearance and have in large part anticipated some of our results. However, the topic is even now far from exhausted; many questions have only been touched upon and others will bear further support. We have, therefore, extended our research along lines not originally contemplated, and the presentation of the experiments in the order in which they were performed would not do justice to the subject. We have accordingly grouped them under various headings.

The tables will take the place of more extended protocols.

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I. TABLES OF EXPERIMENTS.

TABLE I.—EXP. I–XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.

Experiment number.	Other experiments done on same animal.	Animal.	Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected.	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of injected proteid per kilo of animal.
I	(II, III, IV).	Dog ♀	6.13	Vein.	Egg, watery, 1.148%.	1.148	0.7672	0.3825	0.187
II	(I, III, IV), (4 days since last experiment.)	"	"	Hypodermic.	"	......	Only non-coagulable proteid.	......	......
III	(I, II, IV), (6 days since last exp.)	"	"	Vein.	Normal salt, 110 cc. 4 min.	......	.....	Died before excretion was completed.	......
IV	(I, II, III), (6 days since last exp.)	"	"	Hypodermic.	Egg, watery, 4.156%.	2.078	1.402 +		0.338
V	None.	"	5.9	Vein.	Egg, freed from globulin by dialysis, 4.470%.	2.2484	1.463	0.785	0.381
VI	"	"	5.3	"	Egg Syntonin in alk. solution. Proteid = 1.276%. Alkalinity = 0.376% NaOH.	1.914	Anuria to death.	......	0.361
VII	"	"	8.75	"	Alkali alb. from egg. Proteid = 0.243%; Alkal. = 0.116%.	0.243	0.0719 alkali alb. 0.0142 coagulable albumen.	......	0.028
VIII	(X)	♂	6.47	Hypodermic.	Alkali alb. from egg. Proteid = 2.447%. Alkal. = 0.316%.	2.227	None.	2.227	0.344
IX	(XI)	"	10.	Vein.	Egg, watery, 0.055%.	0.0453	0.6817	Negative.	0.0045
X	(3 days after VIII.)	♀♂	6.47	Hypodermic.	" " 5.236%.	4.1364	1.3325	2.8089	0.639
XI	(4 days after IX).	♀	10.	Vein.	Alk. alb. from egg. Proteid = 3.073%. Alkal. = 0.495%.	6.146	Trace, both alk. and native.	6.14	0.615
XII	None.	♂	5.23	Hypodermic.	Egg-albumen.	0.1218	3 days; 0.0135 4th day; 0.5861 and albumose.	......	0.023
XII A	5 days after last.	"	"	"	" " (free from albumose.)	0.0609	Trace of albumose.	0.06	0.0116
XIII	None.	"	4.8	"	" " albumose.)	1st day; 1.5266 2d "; 1.6240 3d "; 1.2992 6th to 9th day; 1.4078 Total, 5.5876	Little albumen, considerable albumose.	......	1.220 in 9 days.
XIV	"	♀	5.7	Vein.	4.742 % albumen + 0.5 % Na₂ CO₃ (unconverted.)	7.7366	Much albumen (urine solid on heating).	Died before complete excretion.	1.356
XV	"	"	2.7	"	Egg in normal salt, 1.955%.	1.7146	Much albumen.	Died before complete excretion.	0.635
XVI	"	"	2.7 (fat)	"	Egg, watery, 2.350%.	1.892	0.7372	1.1546	0.701
XVII	"	"	4.55	"	Undiluted egg albumen, 12.769% (14.8%).	12.769	......	......	2.806
XVIII	"	"	4.5	"	Egg, watery, 2.849%.	2.840	1.5826	1.2574	0.631
XIX	"	"	4.1	"	" "	2.849	1.5646 +	Died before complete excretion.	0.702
XX	"	"	7.7	"	" normal salt, 0.655%.	0.0721	0.2823 in 8 days, and still excreting.	More excreted.	0.009
XXI	"	"	4.8	"	" "	0.0629	0.1771 in 5 days.	" "	0.013
XXI A	in 6 days, 0.2 Gm. Morphine.	"	"	Hypodermic.	......	......	......	......	
XXII	None.	"	9.5	Vein.	Egg, watery, 1.855%.	1.855	[1.4080 in 2½ hours.]	......	0.195

TABLE I.—EXP. I–XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.—Continued.

Experiment number.	Other experiments done on same animal.	Animal.	Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected.	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of proteid injected per kilo of animal.
XXIII A	None.	Guinea.	0.275	Hypodermic.	Egg, watery.	0.3+ in 2 days.	.....	.....	.....
XXIII B	None.	"	0.245	"	"	"	.....	.....	.....
XXIV A	(XXVIII).	Rabbit ♂	2.26	Ear-vein.	"	0.156	Too small to be accurate.	.....	0.069
B	"	♀	2.25	"	"	0.281	Too small to be accurate.	.....	0.125
XXV A	None.	Guinea	0.485	Hypodermic.	"	1st day 0.094 / 3d " 0.031 / 12th " 0.084	.....	.....	In 12 days A 0.307
B	"	"	0.230			Each { Total in 12 days 0.149	.....	.....	B 0.648
XXVI	"	Dog ♂	5.9	Vein.	3.402%	3.402	......	......	0.576
XXVII	"	"	7.0	"	3.4%	5.1	[4.662 in 1 hr. 40 min.]	......	0.729
XXVIII A	(XXIV) 8 days after previous injection.	Rabbit ♂	2.21	Hypodermic.	8.302%	6.469	0.963 +	} Died before complete excretion.	2.927
B		♀	2.05	Peritoneum.	"	5.850	0.931 +		2.853
XXIX	None.	Dog ♂	5.8	Vein.	norm. salt 3.39%	5.096	......	......	0.875
XXX A	"	Guinea Pig	0.33	Peritoneum.	" 1st day / 4th day / 7th day / 8th day	A. 0.203 / 0.763 / 0.569 / 1.138	......	......	In 8 days A 8.119
B	"	"	0.25	"	"	B. 0.203 / 0.636 / 0.569 / 1.138	......	......	B 10.184
XXXI / XXXII	"	Rooster / Rabbit ♂	1.21	Vein.	Total in 8 days. norm. salt. " heated to 73°, 1.255%.	2.673 / 0.763 / 1.673 / 2.546	None. [0.6174 in 1 hr.]	......	1.382
XXXIII	"	Hen	1.79	"	Human muscle in normal salt 0.389%.	0.486	None in 5 hours.	......	0.272
XXXIV	"	"			Egg, watery.	Dies at end of injection.	......	......	......
XXXV	"	"		Peritoneum.	"	Dies as wound is sewn up. 2.275	......	......	
XXXVI	"	Rabbit ♀	0.975	"	4.5%.	1st day 4.31 / 5th " 3.5	0.367	1.908	4.42
XXXVII A	"	"		"	"	1st day 4.31 / 5th " 3.5	0.915	3.305	3.59
B	"	"					0.794	2.706	
							1.387	2.923	
							1.112	2.388	
XXXVIII A	"	"	0.185	"	Chicken muscle in normal salt 4.375%.		Died.		3.64
B	"	♂	1.140	"	"		None.		2.95
XXXIX A	"	"	1.220	"	Dog's muscle norm. salt.		Died.		3.83
B	"	"	0.973	"	"		None.		3.74
XL	"	Dog ♂	0.905 / 3.75	Hypodermic.	Egg, watery.		None.	All.	
XLI	"	♀	3.	Vein.	Egg with equal vol. norm. salt.	0.469	Dies at end of injection.	0.46	0.125
XLIV	"	Rabbit	1.570	"	Dog's muscle in norm. salt.	100 cc.	None.	All.	......

TABLE II.—CONTINUATION OF EXPERIMENTS I-XLIV. PERCENTAGES OF PROTEID RETAINED AND EXCRETED. DURATION OF EXCRETION. EXAMINATION OF URINE.

Experiment No.	Amount of proteid retained per kilo.	Per cent. of the injected proteid excreted.	Per cent. retained.	Per cent. excreted in first 24 hours.	Excretion lasted between—	Special remarks on urine.	Glycosuria.
I	0.062	66.67	33.33	....	42 and 55 hours.	Non-coagulable proteid.	1.3 gm.
II	....	....	....	....	2 and 5 days.	....	None.
III	....	....	....	....	Over 3 days.	....	1.956
IV	0.133	67+	34.93	49.5	43 and 64 hours.	Small amount of globulin.	None.
V	0.344	65.07	....	....	40 hours.	No casts.	"
VII	....	0	100	0	0	0	"
VIII	....	....	....	....			"
IX	....	....	....	....		Contains haemoglobin and globulin, probably from admixture of wound secretion, since this kept open.	"
XI	0.433	32.	68	....	55 and 71 hours.	No globulin.	None.
XII	0.615	0	100	0	0	No casts.	"
XIII	0.021	11	89	....	2 and 3 days.	" "	"
XIV	....	....	....	....	11 days after last injection.		None.
XV	....	87+	....	38.1		....	"
XVI	0.427	38.9	61.1	42.	24 and 42 hours.	*Trace globulin.*	Large amount.
XVIII	0.279	55.7	44.3	40.5	90 hours.	....	Fair amount.
XIX	....	55+	....	167.	42 hours.	....	None 1st day, some 2d, none after, but plenty on morphine.
XX	....	....	....	125.	10 days+.	Much globulin.	None.
XXI	....	....	....	....	5 days.	No albumose.	"
XXII A and B	....	....	....	[75 in 2½ hours.]		Much globulin.	"
XXIV A and B	....	....	....	[137 in 1 hr. 40 min.]		Considerable albumose.	"
XXVI	....	14.9+	....	5.1	3 days+.		....
XXVIII A	....	15.9+	....	13.9	3 days+.		....
" B	....	....	....	....			....
XXIX	....	....	....	[36.8 in 1 hour.]		No globulin; no precipitate below 73°	....
XXXII	....	....	....	....		Proteid free.	....
XXXIII	3.482	16.1	88.9	16.1	24 hours.	No globulin.	....
XXXVI	2.775	21.2	78.8	10.3	3 days.	Albumose increased.	....
XXXVII A	2.467	22.7	77.3	12.7	3 days.	" "	....
" B	2.015	32.2	67.8	17.4	2 days.	" "	....
XXXVIII A and B	....	31.8	68.2	8.1	3 days.	No albumose.	....
XL	0.125	0	100	....	0	....	None.
XLIV	....	0	100	....	0	....	"

TABLE III.—CONTINUATION OF EXPERIMENTS I – XLIV. SYMPTOMS AND AUTOPSIES.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
I	....	Stupid next day, lively on 2d.	....	Diuresis.
II	....	None.	....	Normal saline on sugar.
III	....	Steadily emaciating since beginning of experiment, but voracious appetite.	....	....
IV	3 days after last injection.	Greatly depressed next day, can scarcely walk on 3d. Temperature 35.5° C.	Extensive pyothorax; acute congestion of kidneys; heart muscle normal.	....
V	Killed 5th day.	None. General condition good.	Slight cortical congestion of kidneys, particularly Malpighian tufts; cells granular.	....
VI	7 hours.	Coma and convulsions; bloody diarrhœa.	Hæmorrhage, congestion and necrosis of abdominal organs; blood changes. See p. 251.	Peculiar toxin action.
VII	....	None.	....	Excretion native albumen after injection of alkali albumen.
X	Killed on 3d day after last injection.	None. Good condition.	Congestion of abdominal organs, particularly cortex of kidneys.	....
XI	Killed on 2d day after last injection.	Almost in stupor.	Kidneys mislaid; other organs practically normal.	....
XII	Killed on 7th day after last injection.	None.	Negative.	Transitory albuminuria 4 days after injection.
XIII	About 40th day.	Emaciation.	Not made.	Long continued excretion.
XIV	4 hours.	Progressive medullary paralysis	Negative.	Death probably from embolism; injection of mixture of alkali and albumen.
XV	16 hours.	Not known.	Negative, but stomach distended with 10 ounces (= 10% body weight) of semi-solid food.	Shock during full digestion?
XVI	....	....	....	Coagulation temp. of urine.
XVII	15 minutes.	Respiratory, then cardiac paralysis.	....	Death by embolism.
XVIII	4 days.	None till last hour, then convulsive. See p. 249.	Negative. Microscopically congestion of abdominal organs.	Death under convulsions after 4 days of health.
XIX	40 hours.	Depression, anorexia. See p. 247.	Not made.	Nephritic albuminuria.
XX	....	None.	....	" "
XXI	1 day after morphine.	None.	....	Comparison of fractional heat precipitates. Physiological effects.
XXII	....	....	....	
XXIII A	28 hrs. after second injection.	None for 6 hrs., then depressed.	Much light red fluid in subcutaneous tissue and abdominal muscles. Otherwise negative; kidneys trifle congested.	Toxic effects.

TABLE III.—CONTINUATION OF EXPERIMENTS I–XLIV. SYMPTOMS AND AUTOPSIES.—Continued.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
XXIII B	10 hrs. after second injection.	None for 6 hrs., later not observed. No	Same as XXIII A.	Toxic effects.
XXIV A & B	....	....	....	Metabolism.
XXV A	....	Local ulcers which heal. other effect.	....	No toxic effect.
B	....	None.	....	" "
XXVI	....	....	....	Comparison of fractional heat precipitates. Physiological effects.
XXVII	....	Complete anuria for 4 hours, although 400 cc. of liquid (=56 cc. per kg.) were injected.	....	....
XXVIII A & B	3 days.	Debility and emaciation.	....	Starvation and metabolism.
XXIX	....	....	....	Comparison fractional heat precipitates.
XXX A & B	About 14 hrs. after last injection.	Some depression.	Free fluid in peritoneum; else negative.	Toxic effects.
XXXI	....	None.	....	Rooster. None excreted.
XXXII	....	....	....	Inject. of albumen heated to 73.
XXXIII	5 hours.	Diarrhœa, coma, fall of temperature, respiratory paralysis. See p 247.	Congestion of abdominal organs; degeneration of liver cells.	Muscle inject'n; none excreted. Toxic effects (anæsthetic).
XXXVI	....	....	....	Excretion egg albumen by hen.
XXXVII A & B		None.		Metabolism.
XXXVIII A	Between 5 and 15 hours.	Not observed.	Negative.	Toxicity of chicken muscle.
B	....	None.	....	Retention of chicken muscle.
XXXIX A	....	Not observed.	....	Retention of dog's muscle.
B	Between 5 and 15 hours.	None.	Negative.	Toxicity of dog's muscle.
XL	10 minutes.	None.		Metabolism.
XLI	....	Paralysis of respir'n and heart.		Embolism.
XLIV	Killed on 3d day.	Fever.	Negative.	Myosin. Fever.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
43	Rabbit, 950 gm.	Urethane, 2.5 gm. stomach.	Intravenous injection, ..	Died during injection.	Acute Urethane death.	......	......	......
44	Rabbit, 1570 gm.	Chloretone, 25 cc. sat. sol. stomach.	100cc. Dog's muscle solu. intravenously.	Killed with chloretone on 3d day.	Muscle and death by chloretone.	Kidney: normal. Liver: considerable fatty degeneration of epithelium. Spleen: normal.	Liver: marked in places. Spleen: a few pigment bearing cells.	Liver: none. Spleen: quite profuse.
45	Rabbit.	As 44.	No injection.	Died acutely.	Acute chloretone death.	Liver: marked congestion; cells unaltered. Spleen: normal.	Spleen: slight.	Liver: none.
46	Rabbit.	As 44.	As 45.	Died in 3 to 16 hours.	Subacute chloretone death.	Kidney: greatly congested. Liver: much capillary congestion. Cells generally unaltered. Spleen: normal.	Liver: some.	Liver: none.
47	Rabbit, 2000 gm.	Urethane, 1 gm. rectum.	Intravenous injection.	Died in 1½ hrs.	Acute urethane death.	Kidney: considerably congested. Cells granular. Liver: cells very much vacuolated and granular.	None in liver or kidney.	......
48	Rabbit, 860 gm.	As 47.	"	Died in 3½ hrs.	Subacute urethane death. Urine contained very many granular casts and some blood. Large quantity of free blood in abdomen; clots on removal. (Injury during autopsy?)	Kidney: cells considerably degenerated. Liver: cells greatly vacuolated and fatty.	Kidney: Pigment in the epithelium of the convoluted tubules (iron free.) Liver: some.	Kidney: none.
49	Rabbit, 1310 gm.	As 47.	15 cc. 5 day brooded egg, into jugular.	Died in 5 days.	......	Kidney: granular degeneration of epithelium. Liver: extensive capillary congestion. Thrombi in many veins and capillaries.	Kidney: in epithelium. Spleen: considerable; rather diffuse.	Kidney: none. Spleen: great amount.
50	4 guinea-pigs.	...	Each received 1 cc. of 1 day brooded egg solution subcutaneously.	One dies in 5 days.	......	......	......	......
51 A 53 B 57 D	Rabbit, 1060 gm. 1070 ". 970 "	Urethane, 0.75 gm. rectum.	15cc.(=0.382 gms.) of fresh egg of brooding series. 7 days later. 11cc.=0.254 gm.) of 6 day brooded egg into ear vein, 6 days later, normal salt, subcutaneously.	Killed by medulla stroke, 2 days after last experiment.	......	Kidney: some congestion: cells rather cloudy: no casts. Liver: proliferation of young connective tissue about lobules; Small cells granular. Small Intestine: slightly congested. Cardiac muscle and large intestine: normal.	Kidney: considerable in epithelium of convoluted tubules. Liver: small amount.	Kidney and Liver: none.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
51 B	Rabbit, 1000 gm.	Urethane, 0.75 gm. rectum.	15 cc. (= 0.362 gm.) of fresh egg of brooding series. 7 days later: norm. salt, ear.	Died 7 days after last experiment.	......	Kidney: considerable congestion; cells very granular. Liver: congested; cells somewhat cloudy. Spleen, cardiac muscle and lungs: normal. Hyperleucocytosis in blood of lungs and kidneys, not noticed in other situations.	Small amount in Spleen. None in lungs, cardiac muscle, liver or kidney.	Spleen: some; but most of the pigment does not take it.
54 A	860 gm.	rectum.						
51 C	Rabbit, 1000 gm.	Urethane, 0.5 gm. per rectum.	10 cc. (=0.24 gm.) fresh egg sol. of brooding series, in ear vein.	Killed by medulla stroke, 8 days after last injection.	......	Bacterial infection profound.	Liver: considerable amount of yellow granules in cells. Spleen: large amount, granular and in lumps. Kidney and cardiac muscle: none.	Liver: none. Spleen: large amount; practically all the pigment has taken the stain.
53 A	960 "		10 days later: norm. salt in ear vein.					
56 A	1080 "		2 days later: 15 cc. egg soln. from 9 day brood.					
57 A	1050 "		4 days later: norm. salt intraperitoneally.					
52 A	Rabbit, 1500 gm.	Urethane, 1 gm. per rectum.	20 cc. (= 0.411 gm.) egg soln. of 3 days brood, jugular.	Died in 6 days from starvation.	......	......	......	......
52 B	Rabbit, 1030 gm.	Urethane, 0.5 gm. per rectum.	6 cc. (=0.123 gm., egg soln. of 3 days brood, ear vein. 6 days later: 15cc. alkaline egg syntonin soln. by jugular.	Died in 2 days after last experiment.	Death from bacterial invasion.	Urine contained a few granular casts. Profound bacterial infection.	Liver: only in haemorrhagic areas. Spleen: none.	Spleen: diffuse light blue stain. Liver or Kidney: none.
55 A	980 gm.							
53 C	Rabbit, 1080 gm.	Urethane, 0.5 gm. per rectum.	15 cc. (= 0.347 gm.) egg soln. of 6 days brood, jugular. 2 days later: 6 cc. alkaline egg syntonin soln., ear vein. 4 days later: normal salt subcutaneously.	Killed by medulla stroke, 30 days after last experiment.	......	Strong bacterial infection.	Spleen: considerable. Liver: doubtful. Kidney and cardiac muscle: none.	Spleen: very slight.
56 B	1070 gm.							
57 C	1060 "							
55 C	Dog 4.3 Kg.	Morphine, ether.	25 cc. alkaline egg syntonin soln. femoral.	Killed by heart puncture in 2 days.	......	Kidney: Cells granular; great degeneration in limited areas; casts. Liver: slight cellular degeneration. Suprarenal, spleen, pancreas, small intestine, and cardiac muscle: all normal.	Spleen: some in granules and lumps. Suprarenal: some granules in cortex, normal pigmented cells in medulla. Liver, kidney, all kinds of muscle: none.	Spleen: considerable. Suprarenal: some. Kidney or intestine: none. Liver: diffuse light stain of cells, but granules only in thrombus.
55 B	Rabbit, 1090 gm.	......	10 cc. egg soln. of 9 days brood, ear vein. 4 days later: norm. salt intraperitoneally.	Died 16 days after last experiment.	......	Strong bacterial infection.	Spleen: considerable. Liver: some, Kidney and cardiac muscle: none.	Spleen: very slight.
57 B	1090 gm.							

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
60	Rabbit, 785 gm.	......	6 cc. norm. salt, in ear vein.	Killed by medulla stroke in 3 days.	......	Slight bacterial infection.	Kidney or liver: none. Spleen: considerable amount, granular and clumps.	Spleen: very slight and diffuse.
61	Rabbit, 834 gm.	......	8 cc. norm. salt, in ear vein.	As 60.	......	As 60.	Little in spleen or suprarenal; a few granules in liver-cells. None in kidney.	Spleen: very little. Liver: none.
62	Rabbit, 885 gm.	Urethane, 0.5 gm, per rectum.	25 cc. alkaline egg syntonin into jugular.	Killed by medulla stroke in 10 days,	Long fever.	Kidney: Rather slight granular swelling of epithelium, considerable congestion of capillaries and tufts. Liver: considerable capillary congestion; cells normal. Spleen and suprarenal normal	Spleen: considerable, granular and in lumps. Liver: ditto: Kidney: none.	Spleen: very large amount. Liver: none.
63	Rabbit, 1180 gm.	......	1 cc. alkaline egg syntonin in ear vein.	Killed by medulla stroke in 3 days.	......	Moderate bacterial infection.	Spleen: considerable, Liver: rather large amount of granular.	Spleen: considerable. Liver: none.
64	Rabbit, 1195 gm.	Urethane, 0.5 gm. per rectum.	Died on table.	....	......	Strong bacterial infection.	Spleen: little, Liver: much, granular.	Spleen: weak, diffuse. Liver: a little in spots, diffuse and coarsely granular.
65	Rabbit, 1225 gm.	As 64.	20 cc. alkaline egg syntonin, jugular.	As 63.	......	Very slight bacterial infection: Kidneys: hyaline degeneration in cells of convoluted tubules. Liver: diffuse granular degeneration of cells. Spleen: normal.	Kidney: many granules in epithelium. Liver: considerable free granules. Spleen: small.	Liver: none. Spleen: considerable.
65 A	Dog.	Morphine-ether.	50 cc. alkaline egg syntonin, femoral.	Killed in 3 days.	......	Congestion of abdominal viscera. Small intestine: degeneration of epithelium of villi. Kidney: extensive hyaline degeneration and cloudy swelling. Liver: epithelium normal, also suprarenals and large intestine.	Spleen: considerable, in clumps. Intestine, liver and kidney: none. Suprarenal cortex: slight.	Spleen: considerable. Liver: none. Kidney: some in isolated places in cells.

TABLE IV.—SUMMARIES OF EXPERIMENTS 48 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
66	Rabbit, 1255 gm.	....	2 cc. sol. of rabbit's muscle, in ear.	Killed by medulla stroke in 3 days.	Few bacteria.	*Kidney:* Cells as in 65, but less. *Liver:* considerable cellular infiltration of perilobular tissue. Some congestion, especially of central veins. Blood appears to contain large number of leucocytes. Cells normal. *Spleen and pancreas:* normal.	*Spleen:* small amount. *Liver* and *kidney:* considerable.	*Spleen:* small amount. *Liver* and *kidney:* none.
67	Rabbit, 1380 gm.	....	1 cc. as 66.	As 66.	.....	*Kidney, liver, spleen* and *suprarenal* are all normal.	*Spleen:* very little. *Liver:* some granules in cells. *Kidney* and cortex of *suprarenals:* none.	*Spleen:* almost none. *Liver:* none.
68	Rabbit, 1375 gm.	0.5 gm. urethane, per rectum.	25 cc. soln. of rabbit's muscle, jugular.	As 66.	.....	*Kidney:* extensive cloudy swelling of epithelium of convoluted tubules. Hyaline casts. *Liver:* slightly fatty, otherwise normal. *Spleen* and *Suprarenal:* normal.	*Spleen* and *liver:* considerably granular. *Suprarenal* and *kidney:* none.	*Spleen:* considerable. *Liver:* none.
69	Rabbit, 1485 gm.	As 68.	As 68.	Killed by medulla stroke 10 days.	.....	*Kidney:* some cloudy swelling and vacuolization of epithelium. *Liver, spleen, suprarenal:* normal.	*Spleen:* very little. *Liver:* considerably granular in cells. *Suprarenal cortex:* none.	*Liver:* and *spleen:* none.
70	Rabbit.	....	.....	Killed by medulla stroke.	Normal.	*Kidney:* considerable cloudy swelling of the epithelium of the convoluted tubules. *Liver:* slight congestion, with extensive granular degeneration of cells.	*Spleen:* almost none. *Liver* and *Kidney:* none.	*Spleen:* almost none.
71	Rabbit, 730 gm.	0.5 gm. urethane by rectum.	....	Killed by medulla stroke in 4 days.	Urethane alone.	*Kidney:* very slight degeneration and vacuolization. *Liver:* slight capillary congestion. Cells normal.	*Kidney:* none. *Liver:* slight.	*Liver:* none.
72	Rabbit, 870 gm.	As 71.	.....	Killed by medulla stroke in 7 hours.	As 71.	*Kidney:* some cloudy swelling. *Liver:* extensive granular degeneration, as 70. *Large intestine:* normal.	*Spleen:* considerable. *Kidney* and *liver:* none.	*Spleen:* considerable diffuse pigmentation.

One of us (Sollmann) is responsible for experiments I to XXI, and 44 to 72. The others were done by us jointly. We take this opportunity of acknowledging our obligations to Professor G. N. Stewart, who first directed our attention to this subject, and in whose laboratory the first experiments were made. We are also greatly indebted to Professor Wm. T. Howard, Jr., for the preparation and description of the greater part of the histological material.

II. METHODS.

A. Operation.

A known quantity of egg-albumen, obtained by dissolving egg white in distilled water or normal salt solution (and always containing a small quantity of egg-globulin), was injected under anæsthesia into the femoral vein (dog) or into the jugular (rabbit); or without anæsthesia into the ear vein or intraperitoneally or hypodermatically, and the urine was collected by the methods presently to be described. The dogs were anæsthetized with morphine and ether, using the smallest quantity of the latter which would keep the animal quiet. To insure against albuminuria from the anæsthetic, the urine was drawn and analyzed immediately before injection. The absence of a notable quantity of globulin in typical experiments is also an indication that in these no part of the albuminuria resulted from the anæsthetic. The anæsthesia was performed on rabbits by injecting through a catheter a solution of chloretone into the stomach, or urethane into the rectum. The amounts are noted in the foregoing tables. The injection of the proteid solution was made fairly rapidly through a burette connected with the femoral vein by a glass cannula. It was aimed to make the operation aseptic, but in the case of the dogs we were not uniformly successful in this respect.

The method of collecting the urine differed with the experiment. After intravenous and intraperitoneal injection, the animals were placed in a zinc-lined or galvanized-iron cage, with an opening for the collection of the urine. The cage was washed daily with distilled water and the washings placed with the urine; it was then flushed with a small quantity of saturated boric acid solution, and some of this was also placed in the collecting vessel. The urine collected in this way showed no trace of decomposition. In the metabolism experiments, the animals were catheterized daily and their bladders rinsed with distilled water. These animals were fed purely on oats and water. The dogs received their customary diet of meat, bread and water.

With intravenous injection, the dogs (female) were catheterized before the injection was made, and the catheter was left in position for an hour or two after the injection; the animals being kept on the operating board, lightly under the influence of ether. The wound was then stitched and covered with collodion and the animals were placed in cages for the further collection of urine. In the experiments in which it was desired to know only the ratio of the excretion of the different proteids, thin glass tubes were tied into the ureters and the urine was collected directly from these, the animal in this case not being allowed to recover from the anæsthetic.

The same animals were sometimes used a second time after they had completely recovered from the effect of the first injection. When an animal died, a careful autopsy was made and the kidneys were examined histologically after hardening and sometimes also in the fresh condition. In certain cases, other organs were also preserved in Orth's fluid, sectioned, and stained with hæmatoxylin, eosin, and for iron.

B.   Analytical Methods.

In a majority of the experiments we directed our attention mainly to the total quantity of proteids, but tested in each of experiments I-XLIV for sugar (Trommer's test) and for non-coagulable proteid (ferrocyanide reaction in the absence of coagulable proteid). For further analysis the urine, if not already acid, was treated with 1% acetic acid until it just reddened litmus paper. It was then filtered and the coagulable proteid determined gravimetrically in a definite volume (10-100 cc.). The same method was used for the albumen solution. The urines of experiments I-XLIV were always tested qualitatively and often quantitatively for globulins by Pohl's method (half-saturated sol. ammonium sulphate).

In the experiments in which we intended to weigh separately the precipitates occurring at different temperatures, we heated the urines slowly in a large water-bath and kept them at the desired temperature for $\frac{1}{2}$ hour. If the quantity of material admitted, a separate sample was taken for each temperature. In the observations on metabolism, where we wished to determine the quantity of the various nitrogenous constituents, the small amount of material and the numerous determinations to be made rendered it necessary to institute some preliminary experiments for the selection of a suitable method of analysis.

Preliminary Experiments for the Selection of a Method of Analysis.

1. *Determination of Urea.*—The separation of the different nitrogenous constituents was effected in the first place by phosphotungstic acid in hy-

drochloric solution...As has been shown by various experimenters, the precipitation of nitrogenous bodies by this substance is not perfectly rigorous. The amount of precipitation varies with the time during which the reagent is allowed to act, with the sample of the reagent and with the dilution. The following results are given by the different observers who have investigated this subject. Our own observations are included in this compilation:

TABLE V.—PRECIPITATION OF NITROGENOUS SUBSTANCES BY PHOSPHOTUNGSTIC ACID.

[Where a space is left blank in the table, the substance has not been investigated].

	Mallet (1).*	Reid (2).*	Schöndorff (3).*	Sollmann and Brown.
Precipitated—				
Egg albumen	Precipitate, insoluble in hot water.	......	......	Prec. complete.
Serum proteid	"	......		
Myosin	"		Prec. complete.	
Vitellin	"			
Syntonin	"			
Casein	"			
Legumin	"			
Fibrin	"			
Haemoglobin	"			
Albumoses	"	80 to 90% prec.		Prec. complete.
Gelatin	"		......	
Chondrin	"			
Xanthin series	......	Precipitate.		
Xanthin	......	......		Prec. complete.
Uric acid	......	......	Prec. complete.	" "
Caffein	......	......	" "	
Guanin	......	......	" "	
Lysin	......	"	" "	
Creatinin	"	"		
Ammon. sulphate	......	......	" "	Precipitate 87%. Used Schuchardt's acid. Kahlbaum's precipitates only partially, Merck's completely (Pfandler).
			......	
Creatin	Prec. soluble in hot water.	0	0	
Peptone	"			
Glutamin	"			
Betain	"			
Hypoxanthin	"			
Carnin				
Not precipitated—				
Urea	"	0	0 (absolutely)	0 (absolutely)
Alanin	0	......	0	
Allantoin	0	......	0	
Alloxantin	......	......	0	
Asparagin	0		0	
Aspartic acid	0	0	0	
Glycocoll	......	......	0	
Glycosin	0	0		
Glutamic acid	0	0		
Leucin	0	0	0	
Sarcosin	......	......	0	
Taurin	......	......	0	
Tyrosin	0	0	0	

* These numbers refer to the bibliography at the end of this article.

Phosphotungstic acid allows us to separate therefore:

In solution; Urea, amido-acids and peptones. Since the last two exist only in traces, the filtrate may practically be interpreted as urea.

In precipitate: Proteids (coagulable and albumoses), uric acid, xanthin bodies and ammonia.

The following protocols give the experiments made to test the method. The detail of the tests are the same as given below (p. 222).

1. Phosphotungstic acid + egg-albumen = Precipitate (filtrate free from biuret.)
        "   "   + Peptone (Witte) = Precipitate.
        "   "   + Sodium Urate   =   "
        "   "   + Ammon. Sulphate =   "
        "   "   + Urea   = no precipitate.

2. Urea: the filtrate yields 87% of the calculated nitrogen.

3. Ammonium Sulphate: the filtrate yields 13% of the calculated nitrogen of the ammonia.

4. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 104% of the urea.

5. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 114% of the urea.

It will be seen that the figures for the urea are somewhat in excess.

2. *Determination of Ammonia.*—This was done by Schloesing's Method, which was tested as follows:

1. Parallel determinations on ammon. sulphate solutions give:
    Desiccation..................................... 1.000
    Distillation with sodium hydrate................ 0.925
    Sulphuric acid by barium....................... 0.977
    Schloesing..................................... 0.905
    Hypobromite................................... 0.778 *

2. Schloesing's method on a mixture of ammon. sulphate, urea, sodium urate, peptone (Witte), and coagulable proteid yields 103% of the ammonia.

3. Schloesing's method on another similar mixture yields 107% of the ammonia.

The method gives slightly excessive results.

3. *Determination of Coagulable Proteids.*—This was done by heating the slightly acidulated urine with sodium sulphate to boiling, and then adding a few drops of ferric acetate. A Kjeldahl determination was then done on the precipitate and filter, the nitrogen of the filter being subtracted.

Ferric acetate gives no precipitate with sodium urate or Witte's peptone.

The difference obtained by subtracting the nitrogen of the urea, ammonia and coagulable proteid from the total nitrogen represents the nitrogen of albumoses, xanthin bodies and uric acid, minus the somewhat variable factor of error in the above determinations.

To eliminate this uncertain quantity and to obtain a further insight into the make-up of this rest we tried various methods of directly esti-

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* The decomposition with hypobromite was undertaken with a view of utilizing this method for the determination of the ammonia. It is seen that the result is unreliable.

mating these constituents; but, it must be confessed, with indifferent success.

Our experiments were restricted to such quantities and concentrations as we could expect in rabbit's urine, and we cannot make any statements concerning the usefulness of these methods when employed under other conditions.

4. *Determination of Albumoses.*—We tried the bromine and zinc-sulphate methods (for details see below). The following are our results:

(*a*) *Bromine Method* (Wiley) (4): 1. Mixture of Witte's peptone and uric acid yields 74 per cent of the albumose.

2. Mixture of Witte's peptone, egg-albumen, sodium urate, urea and ammonium sulphate yields 79 per cent of the albumose.

Whilst the method is inaccurate, the comparative values obtained by it are probably useful.

b) *Zinc-Sulphate Method.*—This was tried only in the last series of experiments, but gave the most promising results of all:

1. Peptone (Witte): Completely precipitated; no biuret in filtrate.

Egg-albumen: Completely precipitated; no biuret in filtrate.

2. Sodium urate, ⎫
   Urea,   ⎬ no precipitate.
                  ⎭

(*c*) *Almen's tannin mixture* gave the following results:

1. Sodium urate: Precipitated 71 per cent of the uric acid.

Ammonium sulphate and urea: No precipitate.

2. Sodium urate,   ⎫ were mixed with urea and ammonium sulphate.
   Peptone (Witte),   ⎬ The precipitate with Almen's mixture contained
   Egg-albumen,   ⎭ but 30 per cent of their nitrogen.

This reagent is therefore not applicable.

The methods of directly estimating the alloxur bodies and uric acid appear to require much larger quantities of urine than we had at our disposal, and demand so much time that we did not give them a thorough trial.

The *methods* finally adopted are the following:

1. *Total nitrogen*, by Kjeldahl: 2 to 5 cc. of urine are placed in a 250 cc. flask with 20 cc. of concentrated sulphuric acid, mercury being added in just sufficient amount to aid oxidation, and the mixture boiled until almost colorless. The flask is allowed to cool and the contents rinsed into a litre flask with three portions of distilled water, of about 75 cc. each. A few pieces of metallic granulated zinc are then added, followed quickly by 70 cc. of a 40% (by weight) solution of caustic soda and 10 cc. of 40% solution of sulphuret of potash. The flask is quickly connected with a Liebig condensor, and heated until the distillate is free from ammonia. The distillate is received in a flask fitted air tight to the condensor. This flask contains a measured amount of decinormal sulphuric acid, and is also connected with a U-shaped absorption tube charged with the same. The distillate is then titrated, methyl orange (U. S. P.) being used as an indicator.

A blank determination was made on every new lot of chemicals and corrections applied if necessary.

Duplicate determinations were made in many cases, but not in all.

2. *Phosphotungstic Filtrate.*—A solution of phosphotungstic acid (100 ccm. phosphotungstic acid (Schuchard) to 800 cc. of 4% hydrochloric acid) is added to 5-20 cc. of urine diluted to about 30 cc., until no further precipitate occurs, then made up to 50 cc. and allowed to stand from one to three hours, filtered, and the nitrogen determined as above on 20 or 25 cc. of the filtrate. The result gives the nitrogen of *urea*.

3. *Ammonia* (Schloesing).—20-50 cc. of urine are mixed with 10-20 cc. of milk of lime and placed in a desiccator with a porcelain capsule which contains 10-20 cc. of decinormal sulphuric acid. This is allowed to stand for five days and the excess of acid titrated. The result gives nitrogen of *ammonia*.

4. *Coagulable Proteids.*—50-150 cc. of urine are rendered faintly acid with acetic acid, mixed with an equal volume of 10% sodium sulphate and boiled. When near the boiling point a few drops of ferric acetate are added. This is filtered, the precipitate washed until free from sulphates, and the nitrogen determined in the filter and precipitate. The amount of nitrogen contained in the filter is determined once for all and subtracted. This procedure was used only for the urines collected after injection.

5. *Albumoses.*—*a. Bromine method.*—25 cc. of urine are rendered strongly acid by concentrated hydrochloric acid, 2 cc. of bromine are added, the mixture is shaken, stoppered tightly, and left to stand over night. Some undissolved bromine must remain in the flask. The mixture is then filtered, and the precipitate washed by decantation. The filter is then returned to the flask and subjected to a Kjeldahl determination. Subtraction of the nitrogen of the filter gives the nitrogen of the total proteids. Subtraction from this of the nitrogen of the coagulable proteids leaves that of non-coagulable proteids.

b. Zinc-sulphate method.—25 cc. of urine are saturated with 35 grm. of crystalline zinc sulphate,* and filtered; the precipitate is washed with saturated solution of zinc sulphate and subjected to Kjeldahl, as in (*a*).†

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* It is necessary to use tested zinc sulphate. One commercial sample yielded 2.1 mg. nitrogen per grm., or in a test carried out as above, about 1 mg. for the 25 cc. of urine.

† According to Bömer, it would have been better to acidulate the urine, and the saturated zinc sulphate solution with 2 cc. per 100 cc. of 1:5 sulphuric acid.

By these methods we could determine the following data:

(a) *Total nitrogen*—Method 1.—Exact.

(b) *Urea nitrogen*—Method 2.—Somewhat in excess.

(c) *Non-urea nitrogen*—(a minus b).—Too low.

(d) $\dfrac{Urea\ nitrogen}{Non\text{-}urea\ nitrogen} = \dfrac{b}{c}$—Somewhat in excess.

(e) *Ammonia*—Method 3.—Slight excess.

(f) *Coagulable proteids*—Method 4.—If anything, slight excess.

(g) *Non-coagulable proteids*—Method 5 minus (f).

(h) *Alloxur and uric acid* = a—(b + e + f + g).—Open to too many sources of error to be very reliable, but still of limited value.

III. EXCRETION OF THE PROTEID.

We shall discuss this subject under the following headings:

A. Proportion of the coagulable proteid excreted.

B. What determines the quantity of albumen retained.

C. Nature of the excreted proteid.

D. Excretion of the different constituents of egg-albumen.

E. Duration of the albuminuria.

F. Beginning of the excretion of albumen.

G. Relative quantity excreted in successive periods.

H. Retention of other proteids after intravenous or subcutaneous injection.

A. Proportion of the Coagulable Proteid Excreted.

Older observers, working mainly with qualitative methods, claim that injected egg-albumen is excreted unchanged, almost in its entirety. Thus Stokvis (5) states that egg-albumen given hypodermically " stellt eine durchaus unbrauchbare Substanz dar." But all later experimenters who have investigated this subject quantitatively, agree that this excretion is generally far from complete.

Observations on the quantitative excretion of proteid indicate the need of special precautions to guard against nephritic albuminuria. We have encountered this condition not infrequently. Excluding, therefore, all cases in which the amount excreted exceeded that injected; those in which the albuminuria continued indefinitely; those in which the urine contained considerable globulin; those in which it showed blood-pigment; and finally also those in which the animal died

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before the excretion had ceased, we can present the following results bearing on this question:

After hypodermic injection of very small quantities (the largest 0.125 grm. per kilo. body weight) into either dogs or rabbits, none of the proteid is excreted:

Dogs, hypodermic injection, 0.02 to 0.43 grm. per kg., two cases, retained 68 and 89%.

Dogs, intravenous injection, 0.19 to 0.7 grm. per kg., four cases, retained 33 to 61%; mean, 40%.

Rabbits, intraperitoneal injection, 3 to 4.4 grm. per kg., four cases, retained 66 to 80%; mean, 72%.

The following cases were found in the literature:

Forster (6): Dog, intravenous, large quantity, retained 27.4%.
Lehmann (7): Dog, intravenous, small quantity, retained 23%.
Munk and Lewandowsky (8): Dog, slow intravenous, very small quantity, retained 82%; rabbit, slow intravenous, retained 54%; rabbit, intraperitoneal, retained 68%.

Adding these to ours, we see that, when moderate to large quantities are injected, the *retention* is as follows:

	Dog, intravenous.	Dog, hypodermic.	Rabbit, intravenous.	Rabbit, intraperitoneal.
Number of cases	7	2	1	5
Extremes	23 to 82%	68 to 89%	54%	66 to 80%
Means	35%	78%	....	71%

B.   *What Determines the quantity of Albumen retained?*

From the results given in the preceding section, it is seen that the retention varies from 23 to 100% of the amount injected. It is likewise evident that:

1.   Very small quantities are retained completely, at least when given hypodermically.

2.   With very large quantities (Forster's case) a larger absolute amount is retained, but the proportion of that retained to that excreted is smaller.

3.   The greatest retention occurs on hypodermic injection. Less is retained on intraperitoneal, and least on intravenous injection.

From 3 we may conclude that the *retention varies with the slowness of absorption*, and from 1 and 2 that *the absolute amount retained varies as the quantity injected;* whilst the *proportion retained is inverse to the quantity injected.* A more minute analysis also shows

that, while this is the general tendency, the proportion retained is less readily influenced by the injected quantity, than is the absolute amount retained, differences of 50% in the injection altering the proportion excreted but very little.

A relation can also be made out between the retention and the rapidity and duration of the excretion of albumen. Retention varies with the length of time required for excretion, but inversely to the quantity excreted in the first 24 hours.

Evidently the less the amount of albumen introduced and the slower its absorption and excretion, the more thoroughly is it retained. It may be assumed that the capacity of the organism for its utilization is limited, and these conditions would be the most favorable to it.

However, certain unknown factors also exist, as might be expected. Thus, in experiments XVI and XVIII the same amount was injected in the same manner: One animal retained 44%, the other 61%. But such instances are surprisingly few.

C. Nature of the Excreted Proteid.

The identity of the excreted with the injected proteid has been amply demonstrated for various substances (Munk and Lewandowsky). Stokvis showed by polarimetric methods that this holds true for egg-albumen. We thought it interesting to compare the *fractional coagulation temperatures* of the injected and excreted proteids and found the closest agreement. We often encountered some globulin in the urines; its quantity was ordinarily very small, and could be referred to the traces existing in egg-white. In certain cases a considerable quantity was excreted, but in these there was usually some other indication of nephritic changes.

D. Excretion of the different Constituents of Egg-Albumen.

The hypothesis has been repeatedly advanced that the precipitates which occur in solutions of egg-albumen at 57.5; 67; 72; 76; and 82° C. represent so many distinct chemical entities. Since we have in the kidney an instrument which is evidently capable of separating with great nicety very closely allied proteids, the thought suggested itself that the partial retention of egg-albumen was perhaps due to the complete retention of certain of these constituents, with

complete excretion of others. To solve this problem, we determined quantitatively the ratio of the precipitates at different temperatures in the injected solution and in the urine.

To secure strictly comparable results, both were largely diluted with water. To make the salt concentration the same in both the injected solution and the urine, the latter (previously boiled and filtered) was added to the albumen solution in the same quantity as was used for the estimation of the proteids in the urine itself. The acidity was also carefully brought to the same degree in both. The injection was always made intravenously.

Table VI gives a compilation of the ratios:

TABLE VI.

Ratio of Proteids Precipitating at Different Temperatures.

Degrees Centigrade	Injection. 68:70:73:77		Urine. 68:70:73:77		Serum. 68:70:73:77	
Experiment XXII..	36.4:15.2		56.6:11.1		....	....
	51.6	48.4	67.7	38.3		
Experiment XXVI.	31.8:0	19.4:50.9	27.3:16.2	15.8:40.7		....
	31.8	70.3	43.5	56.5	92.5	7.5
Experiment XXIX.	29.7:9.8	15.6:45.6		29.1:60.3	86.5:8	0 : 5.6
	39.5	61.2	10.6	89.4	94.3	5.6

The figures give the percentage of the total proteid, precipitated at the given temperature.

It is seen that in experiment XXIX, where there was no admixture of globulins, and where serum-proteids can therefore probably be excluded, the lower proteids (68° and 70°) are lessened, *i. e.*, they are retained to a greater extent, or else their coagulation temperature is raised.

Experiments XXII and XXVI, on the other hand, show a relative increase in the lower proteids; but since these contained a large amount of globulins there was an undoubted excretion of serum, and since the latter contains almost purely the lower members, the result is not surprising.

If we calculate the quantity of serum in these urines on the basis, that the serum-albumin amounts to one half of the globulin,—certainly

the smallest possible amount *—and subtract the serum proteids calculated on this basis from the total proteids of the urine, these experiments will also bear out the conclusions formulated from experiment XXIX, the ratios now standing:

	Solution injected.	Urine.
	70° : 77°	70° : 77°
Experiment XXII......	51.6 : 48.4%	34 : 61%
Experiment XXVI.....	31.8 : 70.3%	17.9 : 82.1%

As a converse of this, we investigated in experiment XXX the unabsorbed liquid remaining in the abdominal cavity of a guinea-pig which had died between 3 and 14 hours after an intraperitoneal injection.

	68°	70°	73°	77°
Albumen injected	37.6	25.8	12.	26. %
Peritoneal liquid	53.	13.	21.	12. %

There is a greatly increased proportion of the lower proteid. We did not investigate whether this was due to admixture of serum, or to slower absorption of the lower portion of the egg-albumen.

It having been proved by the above experiments that *the lower proteids are either retained in larger proportion or changed into those of higher coagulation temperature,* we tried whether the higher proteids would be converted into the lower. For this purpose we injected an egg-solution which had been kept at 73° C. for 10 minutes. The urine gave *no precipitate up to 73°,* but a copious precipitation occurred above this temperature. 1.673 grm. had been injected, 0.617 was recovered in one hour.

We consider that this fact lends support to the view that the precipitates occurring in egg-albumen at different temperatures really belong to different proteids; the lower being more completely retained by the body, or converted into proteids of higher coagulation temperature; whilst the higher are less completely retained, and cannot be converted into the lower.

Friedenthal and Lewandowsky (10) have shown that serum loses its toxicity by heating to beginning turbidity (55 to 60°). The above experiments, in showing that excretion occurs when the solu-

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* Cloetta (9) finds in acute nephritis in rabbits produced by aloin that there are 2.37 to 3.71 times as much albumin as globulin in the urine.

tion has been heated to 73°, prove that the non-retention of egg-albumen cannot be due to its content of some such toxin.

In this connection we investigated whether the kidneys of *chicken* also recognized ovalbumin as a foreign proteid.

Since the animals frequently died during or shortly after intravenous injection, the intraperitoneal method was resorted to. The fæces were extracted with water, and this extract tested for proteids in the usual manner.

Exp. XXXI.—Rooster.—Injected solution containing 0.763 grm. egg-albumen. None recovered on two days following.

Exp. XXXVI.—Hen.—Injected 2.275 grm. egg-albumen in solution. On following day the fæces yielded no globulin, but albumen as follows:

68°	70°	73°	77°	total
none	0.1305	0.1230	0.1135	0.3670

The chicken therefore behaves towards the injection of egg-albumen precisely like mammals; the retention of the lower proteids is still more complete.

E.   Duration of the Albuminuria.

In experiments in which no nephritis existed, the albumen disappeared from the urine in:

Mean.

Dogs.   Intravenous injection (4 cases), 1½ to 2½ days.   2 days.
"   Hypodermic   "   (2   "   ), 2 to 3½   "   3   "
Rabbits.   "   "   (1   "   ),   3   "
"   Intraperitoneal "   (5   "   ), 2 to 3   "   2½   "

In 4 experiments on dogs in which nephritis developed (as shown by the excretion of a larger amount of proteid than that injected), the albuminuria lasted from 3 days to several weeks, *i. e.* as long as the animal was kept under observation. Two dogs which excreted non-coagulable proteid after hypodermic injection showed this from 4 to 11 days.

It appears from the above that albuminuria in typical experiments lasts from 1½ to 3 days, according to the manner of introduction. The comparatively long duration after hypodermic and intraperitoneal injection may be referred in large part to slow absorption of the solution.

A large lump persisted for two days at the site of hypodermic injections in rabbits; and, in cases of intraperitoneal injection, proving fatal in three hours or more, much liquid with white flakes was found in the abdominal cavity, although no other sign of inflammation was perceptible.

R. Winternitz (38) found that, after the injection of sterile egg-albumen into the pleural cavity of dogs, the amount of liquid in the pleura had considerably increased beyond the injected quantity in six hours; in sixteen hours, in one dog, it had only fallen to one-half; in another dog there was a trifle more than the injected amount after seventeen hours. The pleural liquid in all three animals contained flakes of coagulated proteid.

The excessively long persistence of a slight albuminuria in a few cases need not necessarily be referred to the injection. Haack (20) claims that the dog's urine frequently contains some albumin. M. Kaufmann (21) quotes F. N. Schulz, that rabbits show a light degree of albuminuria from the most insignificant causes; and Kóssa (23) claims that an idiopathic albuminuria is so frequent that of the numerous rabbits bought in half a year for the physiological institute in Budapest, scarcely two or three were free from it. Others, obtained from a different source, developed it after being caged for a few weeks. The rabbits used by us for quantitative experiments were always free from any noticeable albuminuria.

Our results bear out the statements of other observers on the excretion of egg-albumen. Munk and Lewandowsky (8), after injections into the ear-vein of rabbits, found the albumen mostly excreted on the 2nd day, with perhaps a trace on the 3rd. O. Weiss (11) also found quick excretion after intravenous injection of serum into the rabbit; but on subcutaneous injections he sometimes saw it last for weeks.

Forster (6), using much larger quantities, found all excreted on the 3rd day. Lehmann (7) found the excretion almost complete in one day, except in one case in which there was nephritis.

F. Beginning of the Excretion of Albumen.

The excretion always began quite rapidly. In Experiment I, in which particular attention was directed to this point, traces were made out in seven minutes after injection; the quantity increased gradually, until in 22 minutes the urine became solid on heating.

G. Relative Quantity Excreted in Successive Periods.

TABLE VI.—PERCENTAGE OF THE INJECTED ALBUMEN EXCRETED ON SUCCESSIVE DAYS.

Experiment and amount injected...	Dogs. Intravenous.						Dog. Hypodermic.	Rabbit. Hypodermic.		Rabbit. Intraperitoneal.			
	XIX. 2.849	XVIII. 2.840	V. 2.248	XVI. 1.892	Forster. 73.3	IX. .0458	X. 4.1364	XXVIII A. 6.469	XXVIII B. 5.850	XXXVII A. 4.313	XXXVII Al. 3.493	XXXVII B. 4.313	XXXVII Bl. 3.493
First 24 hours	33⅓% (17 hours)	38.4% (18 hours)	49.5% (23 hours)	38.1%	62%			5.1	13.9	10.3	12.7	17.4	8.1
Second 24 hours	21.6% (17 to 41 hrs.)	14.5% (18 to 42 hrs.)	13.9% (23 to 43 hrs.)	0.9% (24 to 42 hrs.)	8.4%	(48 hours, 13 times, 55 hours)	82%	4.6	14.2	10.3	6.8	13.9	10.4
Third 24 hours	Dead.	2.8% (42 to 67 hrs.)	1.6% (43 to 64 hrs.)	None.	2 %	......	None.	5.1	5.3	1.0	3.0	0.8	7.3
Fourth 24 hours	......	Trace.	None.	......	......	......	......	Dead.	Dead.	0.5	None.	None.	None.

It is seen in Table VI that when the injection is *intravenous* (4 experiments), *about ¾ of the excretion takes place inside of the first 17 hours; the remainder in the next 15 hours, and only traces later.* Where hypodermic and intraperitoneal injections are made, the excretion is more nearly equal on 3 successive days, or may even be higher on the second day than the first. This may be explained by slow absorption.

As to the *quantity excreted in the first few hours*, this must be largely influenced by diuresis—an extremely variable factor.

In one experiment (XXXII) of intravenous injection in a rabbit, in which the animal received 1.663 grm., 37% of the total amount was excreted in one hour. This certainly shows that the process of excretion is a very rapid one.

H.   *Retention of other Proteids after Intravenous or Subcutaneous Injection.*

The recent work of Munk and Lewandowsky (8) has demonstrated the practically complete retention of casein and nucleo-proteid, and the retention of 85% of gelatine. Schaefer and collaborators (22) have shown that considerable proportions of proteoses and similar substances are retained. The complete assimilation of serum-proteids—whether from the same or from foreign species—is almost universally accepted. The common statement is that *alkali-albumin* is also completely retained. We made some experiments on this point.

Exp. VI.—Animal died under anuria.

Exp. VII.—Intravenous, 0.028 grm. per kg. Animal excreted both native and alkali-albumin. Probably some nephritis.

Exp. VIII.—Hypodermic, 0.344 grm. per kg. Urine proteid-free.

Exp. XI.—Intravenous, 0.615 grm. per kg. Urine proteid-free.

Our results therefore agree with those of other observers. We further determined that actual conversion was necessary, for a simple solution of egg in 0.5% sodium carbonate caused the appearance of a large amount of native albumen in the urine (experiment IV).

The statement of the text-books is that *myosin* is not excreted. This appears to rest entirely on an experiment of Lehmann (7) made with frog's myosin. In six experiments we injected extracts of

human, rabbit's, dog's and chicken's muscle into rabbits, intravenously
or into the peritoneum.   In no case was there any excretion.   *The
myosin of foreign species is, therefore, also completely retained.*

IV.   EFFECTS UPON METABOLISM.

From the experiments detailed in the preceding section, it results
beyond doubt that a considerable proportion of the injected egg-
albumen is not excreted in the urine.   Does this leave the body by
any other channel?   If not, is it metabolized in the same manner as
proteid entering the organism by the normal channel?

Semmola (12) states that the injection of egg-albumen causes the
appearance of proteid in the bile.   The original paper was not acces-
sible, but the reference gave no data as to the quantity excreted by
this channel.

In the last metabolism experiment (XXXVII B) it was noted
that the freshly passed fæces on the day following the injection were
rather slimy.   5 grm. were extracted with water, and yielded coagula-
ble proteid corresponding to 0.028 grm. nitrogen for the 16 grm. of
fæces passed in 24 hours.   A glance at the nitrogen of the fæces will
also show that there is a tendency to an increase in the percentage of
nitrogen on the first or second day following the injection.

The quantity due to the injected proteid cannot, however, be calcu-
lated, since the normal variations in the amount of fæces are so great.
Thus, Rabbit A, in 2 periods of 3 days following injection excreted
2.142 grm. N in fæces; on other 6 days 1.774; an *increase* of 0.366 in
the periods after injection.   Rabbit B, after injection 1.467; other days
1.734, a *decrease* of 0.267 for the periods after injection.

We judge from this that the amount leaving the organism un-
changed by way of the fæces must be quite small.   A large amount
remains to be accounted for.   Stokvis (5) states that some is con-
tained in the saliva, but this amount must be very small.

Most observations on the effect of proteid injection upon metabolism
have been made upon serum.   Several of the older authors mention
that this is excreted completely as urea, but we could not learn how
minutely the observations were made.   Forster (6) made observations

on serum and egg-albumen injections; Pflüger (13) shows that in these experiments more urea was excreted than corresponded to the amount injected. Of more recent workers, Arloing (14) finds an increased excretion of N and P_2O_5, but gives no data concerning the relation of the increase to the amount injected. Albertoni (15) finds that the injection of defibrinated blood into the peritoneal cavity of starving or insufficiently nourished dogs caused no increased N excretion, if the animals had previously been well nourished, but increased the nitrogen of the urine if the animals had not been in good condition.

For egg-albumen we can only find the quoted experiments of Forster and statements of Laborde (16) that hypodermic injection into starving rabbits causes an increase of N excretion above the amount injected; but he does not give numerical data in support of his statements.

We decided to investigate this problem, and to give attention also to the form in which the nitrogen is excreted. The analyses for this purpose were made according to the methods laid down in Section II, p. 223. We shall first give a brief account of the experiments and the conclusions to which they lead, and shall follow this with the tables giving the quantitative results from which the conclusions were drawn.

We began our experiments with a dog (experiment XL). Having investigated the changes in metabolism resulting from starvation, and having demonstrated that the hypodermic injection of colloid (gum arabic) was without effect upon metabolism, we introduced subcutaneously 0.125 grm. per kilo. of egg-albumen. Neither coagulable nor non-coagulable proteid appeared in the urine, nor could any alteration in other ingredients be determined with certainty. Precisely the same negative result was obtained by the injection of respectively 0.069 and 0.125 grm. per kilo. into the ear-vein of rabbits (experiment XXIV A and B). We omit these experiments from the tables.

It appeared, therefore, that a much larger quantity needed to be injected to yield conclusive results.

We made two observations on starving rabbits (experiment XXVIII), and two (experiment XXXVII), of two injections each, on rabbits having a free supply of oats, the amount of the latter being weighed each day, and the quantity of N in the food being calculated from this.

It was found impossible to maintain a nitrogen equilibrium. In the case of the starving animals there was a rapid starvation-increase which had to be allowed for. In the fed animals, the injection produced a marked loss of appetite, so that the nitrogen balance had to be calculated from the income and output.

The results show that a considerable part of the injected proteid leaves the body unchanged. Another, smaller, portion is excreted as non-coagulable proteid. The percentage of both was much smaller in the starving animals, but this was probably because less was injected. A certain amount was excreted by way of the fæces.

Taking the rabbits of experiment XXXVII, as showing the minimum retention we have:

Of the 1.249 grm. of N (as egg-albumen) injected into the peritoneum in two doses, 5 days apart,

	Rabbit A.	B.
excreted unchanged in urine,	22.3%	32.7%
" as non-coagulable proteid in urine,	18.7%	25.5%
" as coagulable proteid in fæces, about,	5. %	5. %
Total excreted as proteid,	46. %	63. %

The remaining 54 and 37% (0.675 and 0.463 grm. of nitrogen) must have been either retained or metabolized.

It is seen from the figures that the excretion of the total nitrogen is invariably increased, and generally beyond the amount injected, so that not only is none of the proteid retained, but its injection causes the animal to lose also body-nitrogen. On this account it is not possible to state in what time this excretion of the metabolized egg-albumen occurs.

As to the form in which this is finally excreted, it is seen that the ratio of total nitrogen (excluding that of the proteids) to urea and ammonia is not changed, so that we must assume that the metabolism ends in the normal products, *i. e.* mainly urea.

TABLE VII.—METABOLISM—RESULTS OF EXPERIMENT XXVIII.

Two Rabbits were used: A ♂ 2460 grm. on July 1; B ♀ 2310 grm. They had been kept on an unlimited diet of oats and water for 10 days previous to July 2, 1900, when oats were withheld. The observations were made at 1 P. M.

	RABBIT A.						RABBIT B.					
	July 6.	July 6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.	July 6.	6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.
Day of starvation	3d.	4th.	5th.	6th.	7th.	8th.	3d.	4th.	5th.	6th.	7th.	8th.
N in faeces	....	0	0	0	0	1.105	....	0	0	0	0	0.680
N in urine, total	....	1.663	1.890	2.687	3.328	1.747	....	1.383	2.844	2.468	3.259	2.053
" urea	....	1.313	....	2.233	3.166	1.663	....	....	2.664	1.900	2.849	1.985
" non-urea	....	0.350	....	0.434	0.162	0.084	....	....	0.180	0.568	0.410	0.118
" ammonia	....	0.011	0.010	0.090	0.031	0.037	....	....	0.010	0.004	0.019	0.041
" non-coagulable proteid (bromine)	....	0.151	0.110	....	0.137	0.113	....	....	0.158	0.180	0.129	0.075
" coagulable proteid	....	....	....	0.053	0.048	0.053	....	....	....	0.126	0.133	0.050
Per cent. of total N of urine in urea	....	79	....	84	95	95	....	....	93	77*	88	94
Ammonia	....	0.7	0.5	1.1	1.0	2.	....	....	0.4	0.1	0.6	2.
Non-coag. proteid	....	9.0	6.0	....	4.0	7.	....	....	6.	7.4	4.	4.
Weight = % of original weight	89.8	87.4	84.6	83.3	78.5	65.0	88.7	87.2	82.3	79.4	71.9	56.5
N loss = % of weight loss (exclusive of coagulable proteid)	....	2.77	2.70	3.89	2.77	0.5	....	4.64	2.47	3.53	1.91	0.7

Rabbit A, 8 to 9: Albumen = 1.035 N injec. subcutaneous-ly at 3.15 P. M. of 8th. 9th, Large lump at site of injection. — 9 to 10: 10th, Lump almost gone. — 10 to 11: A. M. of 11th. Found dead at 8.15

Rabbit B, 8 to 9: Albumen = 0.986 N injected into perito-neum at 3.15 P. M. of 8th. — 10 to 11: A. M. Found dead at 8.15

* = 81% of total N exclusive of proteid.

TABLE VIII.—METABOLISM OF RABBIT XXXVII A.

♀ 975 grm. Fed on oats. Observations made at 2 P. M.

July and August, 1900	23 to 24.	24 to 25.	25 to 26.	26 to 27.	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to August 4.
Total N consumed	1.047	0.261	1.396	0.863[1]	0.610	0.575	0.558	0.698	0.908	0.872	0.767	0.715	8.961[1]
" excreted	...	...	1.356	1.312	1.088	0.363	0.364	0.564	0.835	0.597	0.654	0.496	7.579
Total N gained	+30	...	+0.040	−0.449	−0.428	+0.212	+0.194	+0.134	+0.073	+0.275	+0.113	+0.219	+1.382
Weight gained (grm.)	−30	−30	+40	+5.	−70	+25	−20	−10	+35	−5.	+15	+5.	+20
Difference in N = % of difference in body weight	...	...	0.1	Neg.	0.6	0.85	Neg.	Neg.	0.21	Neg.	0.8	4.0	6.9
N in faeces	0.260	0.341	0.600	0.388	0.681	0.095	0.112	0.315	0.394	0.250	0.333	0.146	...
" urine	...	...	0.756	0.924	0.857	0.268	0.252	0.249	0.441	0.347	0.321	0.350	...
N in urea	...	...	0.728	0.746	0.202	0.235	0.229	[0.210]²	[0.270]²	[0.230]²	0.275	0.322	...
" non-urea	...	...	0.028	0.178	0.155	0.033	0.023	0.073	0.137	0.099	0.046	0.028	...
" ammonia	...	...	0.035	0.010	0.007	0.009	0.012	0.025	0.022	0.028	0.008	0.003	...
" non-coagulable proteid (ZnSO₄)	...	...	...	0.048	0.072	0.011	0.007	0.006	0.083	0.045	0.025	0.037	...
" rest (theoretically alloxur, etc.)	...	...	...	0.049	0.006	0.006	0	...	Neg.	...	Neg.	Neg.	...
" coagulable proteid	...	...	...	0.071	0.070	0.007	0.004	...	0.071	0.038	0.018	...	0.279
% of total nitrogen of urine (exclusive of proteids) in Urea	...	...	96	89	79	90	92	...	71.³	78.³	88.	92.	...
Ammonia	...	...	4.6	1.2	2.5	3.4	4.8	10.	6.0	9.	2.7	0.9	...
Non-coagulable proteid	...	...	...	5.5	25	4.2	2.8	2.	22.	12.	8.	11.	...

At 2.45 of 26th N into peritoneum. albumen = 0.690

At 2.37 of 31st N into peritoneum. albumen = 0.559

¹ Including injected.   ² Some urine lost, not over 10%; no correction applied.   ³ Direct analysis missed; estimated by difference.

TABLE IX.—METABOLISM OF RABBIT XXXVII B. ♀ 1185 GM.

Observations made at 2 P. M.

July and August, 1900....	23 to 24.	24 to 25.	25 to 28.	26 to 27.	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to Aug. 4.
Total N consumed	1.134	0.872	0.959	0.951[1]	0.523	0.872	1.221	0.959	0.646[1]	0.785	0.802	0.593	10.315[1]
" excreted	1.352	0.894	1.213	1.494	0.981	0.559	0.631	0.715	0.750	1.093	0.693	0.743	11.118
Total N gained	−0.218	−0.022	−0.254	−1.233	−0.458	+0.313	+0.590	+0.244	+0.663	−0.310	+0.109	−0.150	−0.803
Weight " (grm.)	+10	−10	0	0	−40	+25	+40	−15	+5	−30	+5	−15	−25
Difference in N = % of difference in body-weight	Neg.	0.22	....	.....	1.1	1.2	1.5	Neg.	Neg.	1.	2.	1.	3.2
N in faeces	0.468	0.290	0.230	0.358	0.178	0.259	0.201	0.306	0.292	0.207	0.173	0.239	
" urine	0.884	0.604	0.983	1.136	0.803	0.300	0.430	0.409	0.458	0.886	0.520	0.504	
N in urea	0.805	....	0.917	0.941	0.566	0.260	0.396	0.390	[0.388][3]	0.773	0.454	0.409	
" non-urea	0.079	....	0.066	0.195	0.237	0.040	0.034	0.019	....	0.113	0.066	0.035	
" ammonia	0.011	0.006	0.005	0.008	0.012	0.008	0.005	0.014	0.010	0.009	0.011	0.008	
" non-coagulable proteid (ZnSO₄)				0.067	0.125	0.011	0.010	0.011	0.063	0.097	0.022		
" rest (theoretically alloxur, etc.)				0	0.004	0.005	0.010	Neg.	....	Neg.	Neg.		
" coagulable proteid				0.120	0.096	0.006	0.009	....	0.056	0.072	0.050	....	0.409
% of total nitrogen of urine (exclusive of proteids) in													
Urea	91	...	93	84	80	87	94	97	84	95	97	93	
Ammonia	1.2	1	0.5	0.8	1.7	2.6	1.2	3.5	2.5	1.1	2.3	0.6	
Non-coagulable proteids	...	...	....	6.0	17.6	3.6	2.4	2.7	13.2	11.9	4.7		

At 2.30 of 26th injected Albumen = 0.690 N into peritoneum.

At 2.40 of 31st injected Albumen = 0.559 N into peritoneum.

[1] Including injected. [3] Direct analysis missed; estimated by difference.

The presence of *non-coagulable proteid* as the result of hypodermic and intraperitoneal injections is an interesting fact. It was established by the ferrocyanide reaction after boiling, as also by the bromine and zinc-sulphate methods, so that it seems beyond doubt that these injections may cause the excretion of a substance having these properties. The amount was not sufficiently large to permit further characterization.

No such substance appeared in the case of any of the intravenous injections in which it was sought; nor did it occur in all cases of hypodermic injection. Its excretion sometimes persisted for a very long time—especially after repeated injections. In experiments XXXVII A and B, in which it was estimated quantitatively, the amount was proportional to the amount of coagulable proteid excreted (A: coagulable = 22.3% of the injected, non-coagulable = 18.7%. B: coagulable = 32.7%, non-coagulable 25.5%). There was no suppuration in these cases, so that this theory of its origin may be discarded. The fact that it did not occur on intravenous injection also disposes of the theory that it might result from the fever.

We believe that these albumoses represent a transition stage in the metabolism of the injected proteids. This would be in line with the observation of Krehl and Matthes (17) that deutero-albumoses are formed throughout the fever period, when there is also an increased proteid metabolism. The fact that it is not seen after intravenous injection can perhaps be explained by so much proteid being excreted unchanged that the organism may metabolize the small residue completely. This agrees with the fact that none is excreted after very small hypodermic injections. The fact of its long persistence in some cases favors the view that albumen injection may cause a long persisting alteration in proteid metabolism.

Experiment XXVIII gives good illustrations of the effect of *starvation on rabbits*. This subject has been investigated by Rubner (18), and more recently and thoroughly by Heymans (19), who draws his observations from 32 full grown rabbits, after withdrawal of both food and water. Our observations, which we present in the form of Curve I, show agreement with the latter observer as regards loss of weight (35 and 43.5%), the curve of loss of weight declining suddenly on the last day; and in the premortal rise of nitrogen

excretion.   We wish to call particular attention to the slight change
in the nitrogen during the first four days.

CURVE I.
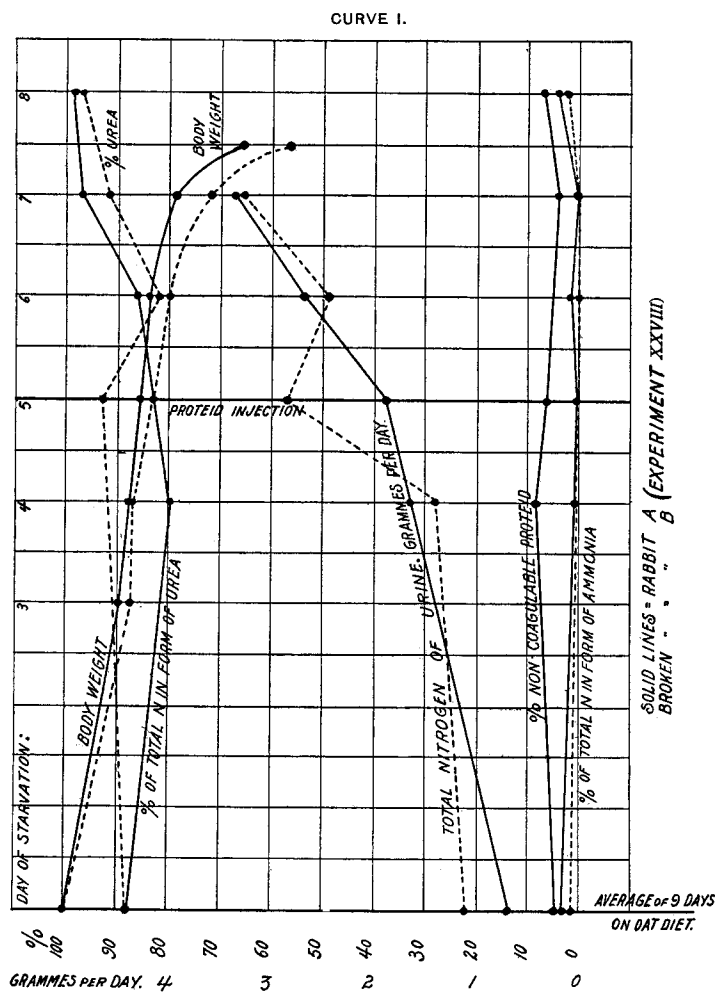
This agreement is remarkable in view of the fact that our animals
were supplied freely with water.   On the other hand, death occurred
between the 7th and 8th day, whereas in Heymans' experiment it hap-
pened on the 6th to the 26th, averaging 15 to 16 days.   That the early
fatality might be connected with the proteid injection seems very im-
probable, since the loss of weight is so nearly the same as in Heymans'

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observations. Some of Rubner's animals (18) also succumbed as early as the 4th day. Other instances are quoted by Kaufmann (21). We are inclined to refer it rather to the fact that our animals were young, and that their metabolism therefore was more active. The early occurrence of the starvation-rise in the nitrogen excretion, points in the same direction.

A striking feature in our cases is the fact that the proportion of urea to total nitrogen is notably increased in the last days of starvation.

Heymans also noted that it was higher during starvation than before, but since he made the urea determinations by a rather rough hypobromite test, he did not feel himself justified in laying stress upon the small difference. We believe that our methods are sufficiently trustworthy, and although the number of experiments is very small, yet they seem to justify the conclusion that the nitrogen metabolism in starvation tends to more complete oxidation than normally. This tends to support Voit's explanation of the cause of the premortal rise in nitrogen excretion, *i. e.*, that it is due to the body becoming relatively poor in fat; as against F. N. Schulz (24), who accounts for it by more proteid being thrown into the circulation by the extensive dying of cells; or another theory, which supposes that the cells become incapable of utilizing fat. With either of the last two theories, one would rather expect the oxidation of the proteids to be less complete.

V.   PHYSIOLOGICAL AND OTHER PHENOMENA.

These will be considered in the following order:

A.   *Effects in Non-Fatal Cases.*

1. Respiration and Circulation.
2. Temperature.
3. Diuresis.
4. Glycosuria and Hæmoglobinuria.
5. Histological Findings after Injection of Egg-Albumen.

B.   *Fatalities Occurring in the Experiments and Their Causes.*

Anæsthetics.
Bacterial Infection.
Toxic Effects of Egg-Albumen.
Toxic Effects of Alkali-Albumin.
Myosin.

A. EFFECTS IN NON-FATAL CASES.

1. *Respiration and Circulation.*—During the earlier experiments on intravenous injections it was always noticed, incidentally, that the respiration increased. In two experiments (XXII and XXVI) special attention was given to this phenomena; tracings were also taken from the carotid-artery and the venous pressure was measured by a water manometer connected with the central end of the femoral vein. In one animal the injection of egg-solution was preceded by an equal amount of normal saline, to allow of a comparison of the two. It was found that the effects were essentially the same in both cases. The respiration was deepened, the heart slowed and strengthened, both the arterial and venous pressures showing a small rise persisting through some 15 minutes. It may, therefore, be stated that intravenous injection of egg-albumen has no effects upon respiration and circulation beyond those of the liquid with which it is introduced.

This result differs considerably from that obtained with sera of foreign species (Arloing (14), Weiss (11), Friedenthal and Lewandowsky (10), &c.). Thus Arloing (14) describes after injection of horse serum into dogs a stimulation of the respiratory centre and a depression of the cardiac muscle and vascular tone. T. G. Brodie (25) finds that the intravenous injection of serum of any source into cats causes arrest of respiration, inhibition of the heart, and vasodilation. All these phenomena last for some time and are produced reflexly by stimulation of the pulmonary branch of the vagus. The active substance is an albumin, coagulating at 86° C., produced during the clotting of the blood. Repetition of the injections leads to immunity. These results are obtained only on the cat. Nothing of the kind was ever seen by us with egg-albumen or its modifications or with muscle on dogs or rabbits.

It must be remembered that the proteid content of our solution was only ¼ to ½ as strong as that of serum. Stronger solutions are impracticable, since a proteid content of 10 to 15% causes speedy death from embolism, preceded by dyspnœa, then respiratory paralysis and lastly cardiac stand-still. Two deaths from this were recorded (experiments XVII and XLI). Another death (experiment XIV) from what was probably embolism from injected air occurred in four hours after injection, with symptoms of progressive medullary paralysis.

2. *Temperature.*—The statement is found in text-books (Lazarus-Barlow, General Pathology) that injections of proteids—particularly

albumoses, but also others—cause a rise of temperature. Quincke (26) notes this for the intravenous injection of defibrinated blood. Injection of other substances may also cause hyperpyrexia. Thus E. Haack (27) noted it after the subcutaneous injection of silver nitrate and tincture of iodine; these substances, however, cause simultaneously an albumosuria. Combemale and Mouton (28) found that the hypodermic injection of 20 cc. of normal salt solution produced a rise of temperature fairly regularly in tuberculous patients, but not in healthy individuals, nor in those suffering from other diseases. For animals, however, Thompson (29) has shown that the intravenous injection of small quantities of normal saline caused a rise of temperature to 2° C. Bosc and Vedel (30) have seen the same effects from larger injections.

In a number of our experiments on rabbits we directed particular attention to the effects of various injections upon the temperature. A self-registering thermometer was inserted for a depth of 7 cm. into the rectum, and left in position until no further rise occurred. The results are represented in Curves II on Plates XVI-XIX. The arrow indicates the time of injection. Urethane was given in all the cases of intravenous injection

It will be seen that all the proteids which were tried (albumen, derived albumen and myosin) produce a very distinct rise of temperature. This occurs whether they are injected subcutaneously, or into the peritoneum, the ear-vein, or the jugular vein. In the last, the rise is often masked by the fall of temperature due to the anæsthetic.

When the amount of the latter was very large, and especially when it was fatal, it prevented the rise entirely. Urethane alone, 0.5 grm. per kilo. by rectum, lowered the temperature 2.3° C. The existence of the bacterial infection, presently to be described, had no influence on the fever, either as to its height or duration. All the proteids had about the same effect.

The rise begins in about an hour after the injection of the solution, and reaches its maximum in from 6 to 24 hours (usually 6 to 8 hours). It then falls very rapidly, but in rare cases may persist for 2 or 3 days (Experiment 62, Plate XIX). A recurrence of the fever is

CURVES II.
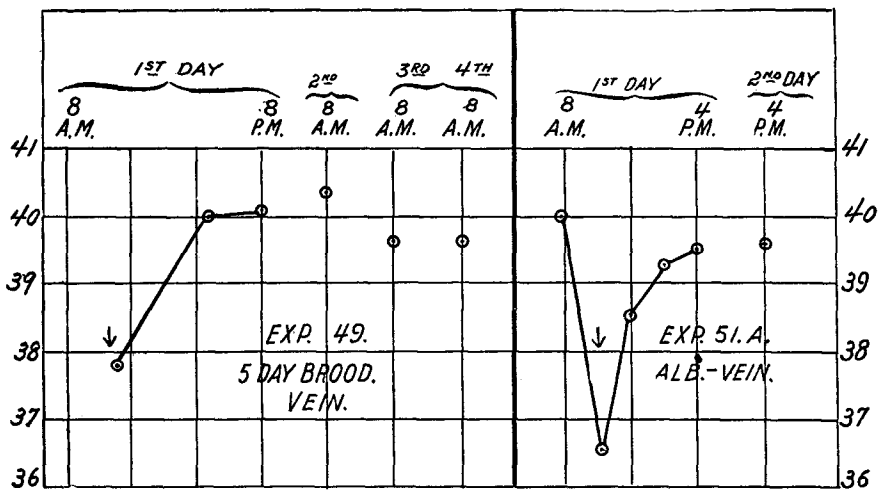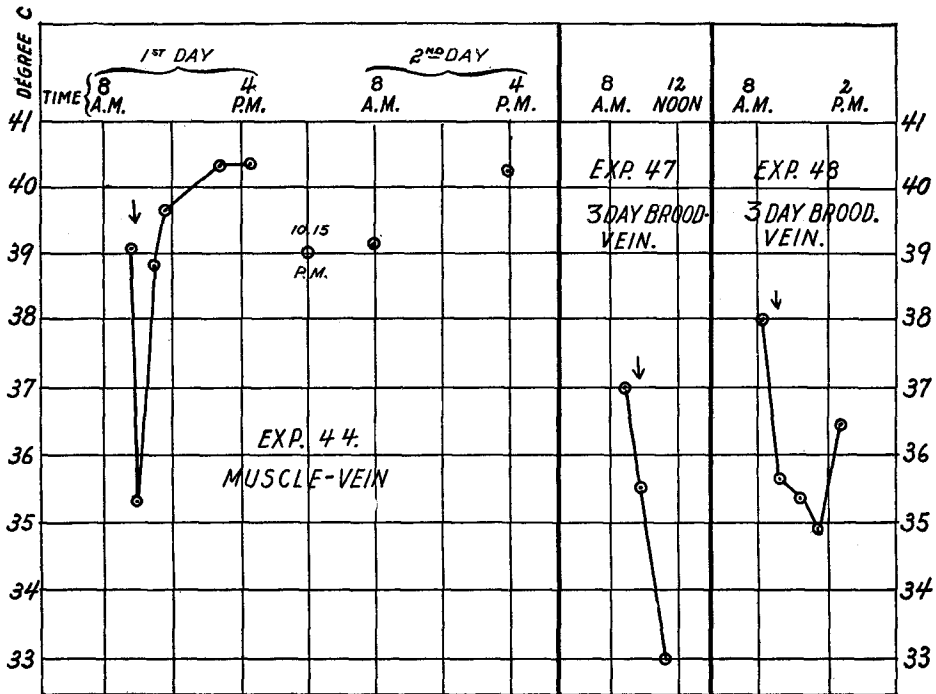
CURVES II—Continued.
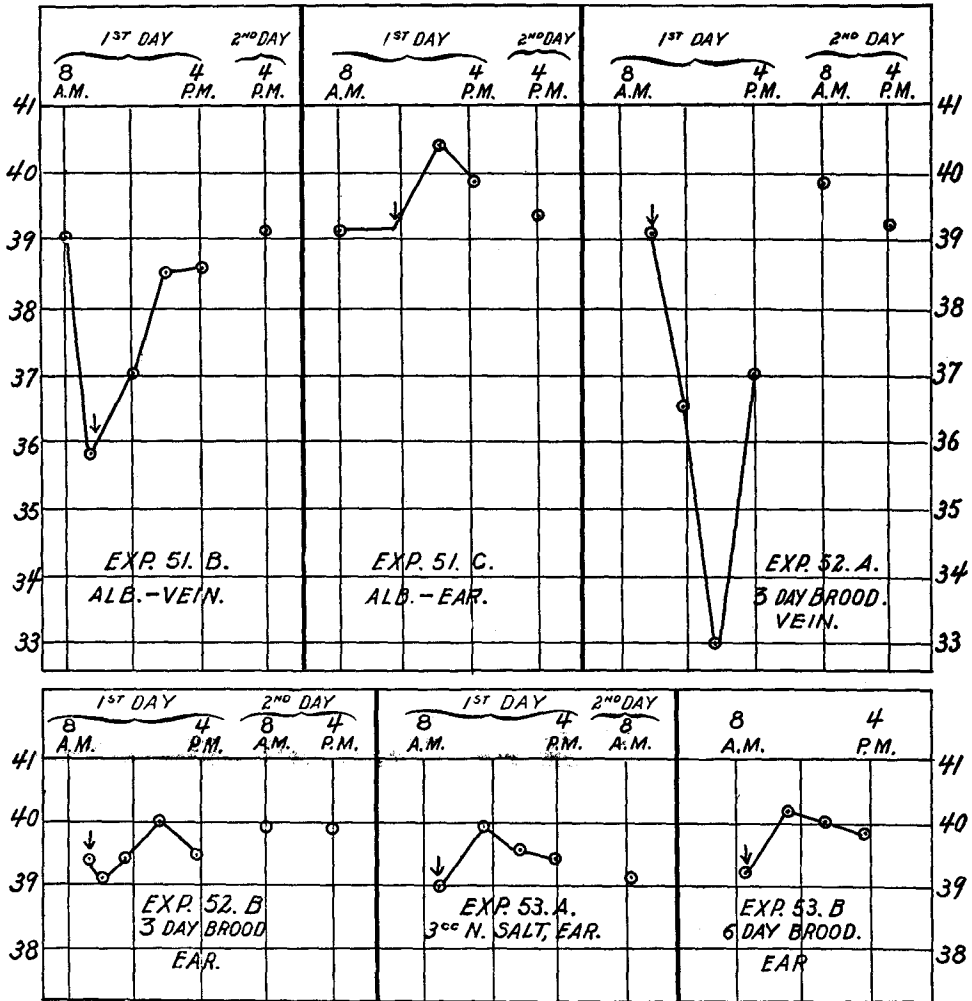
CURVES II—Continued.
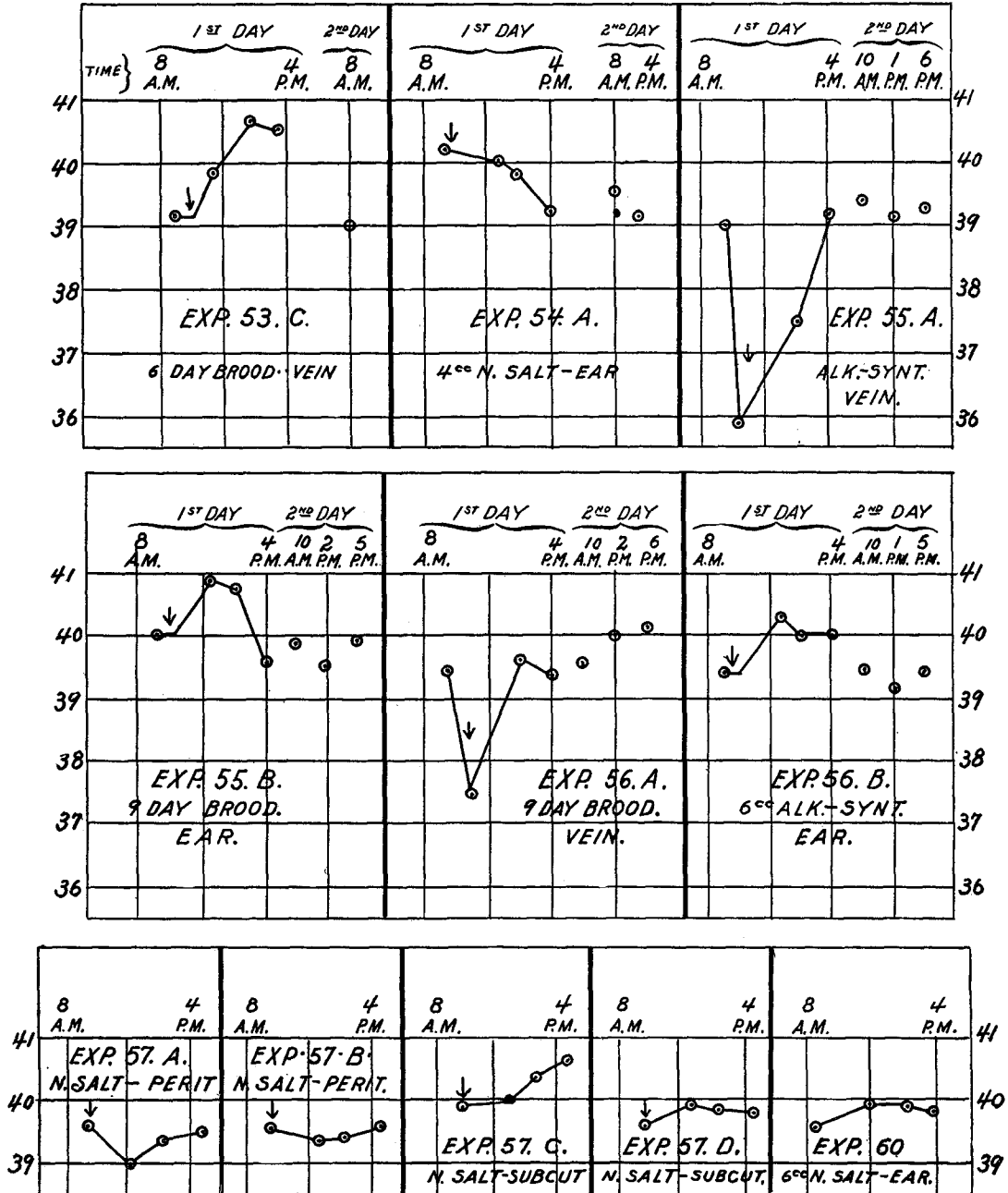
CURVES II—Continued.
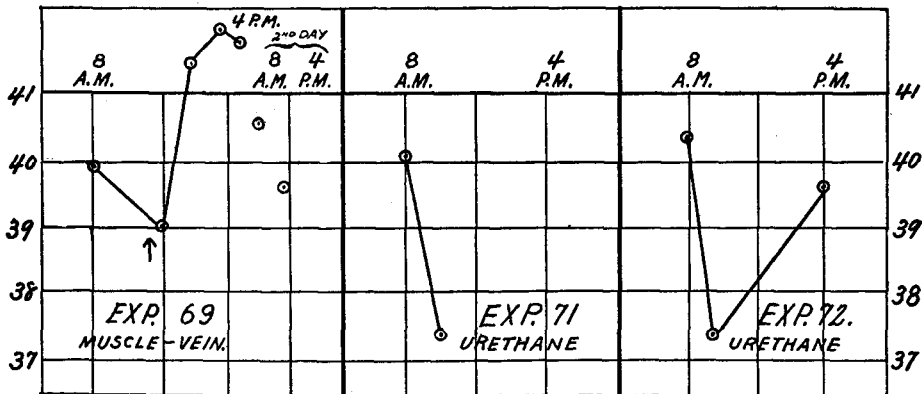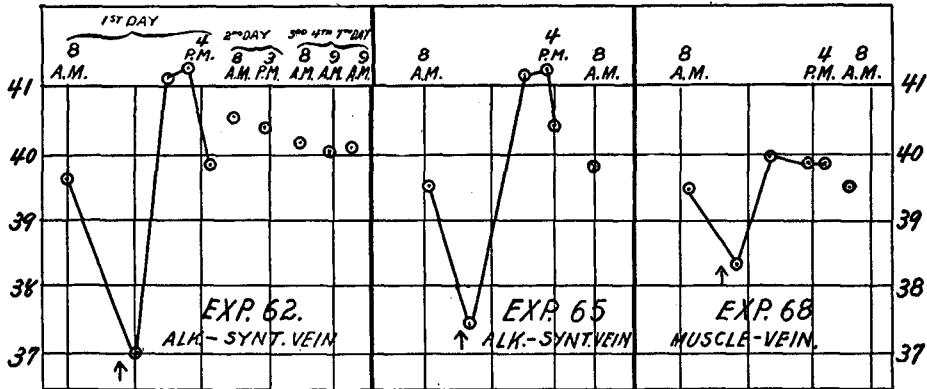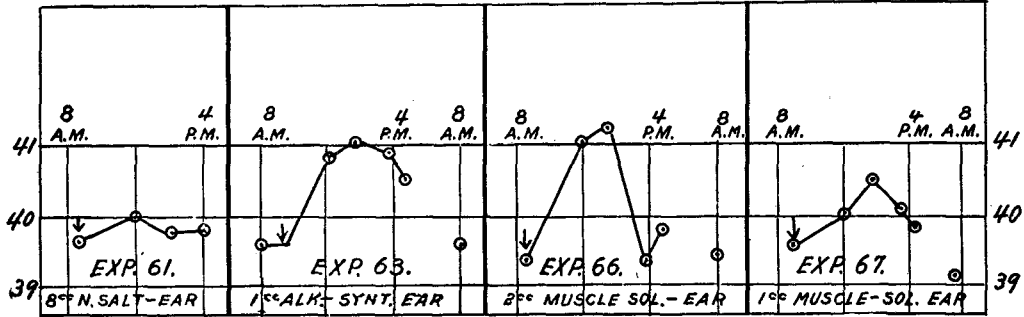
sometimes seen on subsequent days, particularly in the afternoon. The rise is usually a trifle over 1°, but may be as much as 2° C.   It seems to be almost independent of the quantity injected; at least, it may be produced by extremely small quantities (as in experiment 66 by 15 mg. of the proteid of rabbit's muscle, per kilo, of injected rabbit).

Normal salt solution alone, when injected into the ear, may cause some rise of temperature, but smaller than that following the use of the proteids (the mean being 0.35° C.). Subcutaneous injection may also cause a rise, but this is still smaller. In some cases the injection of normal salt solution, by way of the ear, skin or peritoneum, causes a slight fall of temperature (from reflex dilatation of the vessels?).

3. *Diuresis*—The injection of egg-albumen tends to cause a diuresis.   In certain animals an anuria persists for many hours, but this may be seen without albumen injection, and even after the injection of a third of the body-weight of normal saline.   The curve of the diuresis, as recorded in two cases, differs very greatly from that produced by the injection of normal salt solution (Sollmann 31).

With the latter the flow of urine reaches its maximum in $\frac{1}{2}$ hour and has returned to a relatively small figure in $1\frac{1}{2}$ hours.   With albumen injection, on the other hand, there is a small primary rise, reaching its maximum in 15 to 20 minutes, and then a fall to 50 minutes.   This curve is probably attributable to the salt solution which carries the proteid, since it is practically identical with that produced by salt solution alone.

About 50 minutes after the injection, however, a second diuresis sets in, reaching its maximum in about 2 hours, when it is 3 to 5 times greater than the primary rise.   This must be due to a specific irritation of the kidneys by the albumen.   We did not investigate whether this action is exerted upon the vessels or cells.   The accompanying Curve III, from experiment I, gives a typical illustration of this phenomenon (Plate XX).

4. *Glycosuria.*—This never occurred on hypodermic or intraperitoneal injection.   It was noted in the case of dogs, after intravenous injection, in 5 out of 18 animals.   The quantity was deter-

mined in but two experiments (I and III). In the latter it reached
0.32 grm. per kilo. The excretion was completed inside of the
first 24 hours, except in one experiment (XXI) in which it appeared
on the second day after injection, the urine being free from it on
the first. In this animal, morphine alone caused an abundant gly-
cosuria.

Evidently the excretion of sugar must be referred to the anæsthetic
and to the morphine. Injection of the proteids does not cause gly-
cosuria.

Hæmoglobinuria.—This was observed in only one case (experiment
XII) and could not be attributed to the injection of albumen. The
urine remained normal for 7 days after a second hypodermic injection,
when it suddenly showed oxyhæmoglobin and methæmoglobin, a
strong content of albumin, but no casts. On the following day these
had disappeared, but the urine now contained bile pigment. The
animal was sacrificed on this day. The autopsy was negative, and
microscopic examination of the kidneys did not reveal anything
abnormal.

In one experiment on a rabbit (No. 48) the bladder at autopsy was
found distended with a reddish urine, giving the oxyhæmoglobin spec-
trum. Microscopic examination showed the abundant sediment to con-
sist of numerous red blood-cells and a large quantity of granular casts.
These appearances were not present in any other experiment of the
entire series. The animal, which had received some albumen-solution
intravenously, had died 3½ hours after the injection, evidently from the
effects of an overdose of the anæsthetic (urethane, 1.18 grm. per kilo.;
temperature fell to 35° C.). The peritoneum contained a large quan-
tity of free blood, which clotted on removal. A rupture of the liver
was noted, but it could not be decided whether this was inflicted at the
autopsy (the liver tissue being extremely friable); or whether it had
occurred earlier, perhaps during the injection. The microscopic exami-
nation showed considerable degeneration of the renal and hepatic epi-
thelium. The last was certainly due to the urethane. The urine,
which was of neutral reaction, showed a large quantity of coagulable
proteid, but no sugar. This fact shows that the albumen was not due
to the hæmorrhage, but that the excretion of the injected albumen was
well advanced at the time of death. The marked nephritis, shown by

CURVE III.
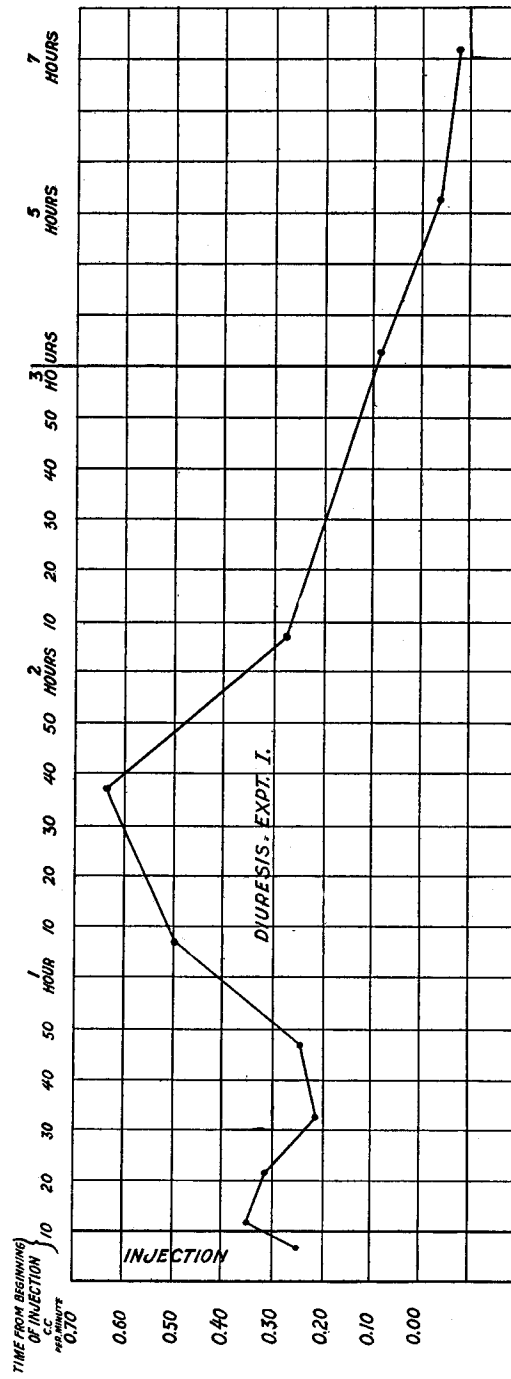
the casts and by the histological appearances, can not have been due to the injection. Nor do we think that it could have been caused by the urethane, and we must leave it unexplained.

5. *Histological Findings After Injection of Egg-Albumen.*

Previous experimenters have found that the injection of proteids causes slight, but distinct, nephritic changes. Semmola (12), Laborde (16) and Seigert report parenchymatous nephritis after injection of egg-albumen. Laborde also mentions necrotic foci in the liver; Semmola reports retinal changes. O. Weiss (11), injecting foreign serum into dogs, finds only hyperæmia of the renal cortex. A. Pettit (32) remarks that the injection of eel serum causes in rabbits a rapidly progressing degeneration of renal epithelium, noticeable even when the animal survives only a few minutes.

We were able through the kind assistance of Professor Wm. T. Howard, Jr., to subject the greater part of our material to histological examination. The methods employed have been described in Section II (p. 218). The animals were killed at various times, but usually several days after the injection.

The *kidneys* of nearly all the animals examined showed a greater or less degree of congestion of both cortex and medulla. Those of the dogs, after injections of plain albumen, presented no noticeable degeneration. In the rabbits on the other hand, the epithelium of the convoluted tubules was almost always cloudy and swollen, sometimes even hyaline or vacuolated. Granules of iron-free pigment were seen in the epithelial cells in many cases. Hyaline casts, resulting from coagulation of the albuminous urine, were often seen in the tubules. These changes, except the last, are such as we often saw in animals which had received no injections. It is quite possible, though by no menas certain, that they are more intense after the injection of the proteids.

In experiment 48 alone, described on page 244, was the degeneration at all extensive. This was also the only case in which casts were at all numerous in the urine. The cause of the peculiarities of this experiment must stand unexplained.

We may say, then, that the injection of egg-proteids does not lead to any conspicuous nephritic lesions. This holds true for dogs, rabbits and guinea-pigs. A slight degree of cellular degeneration cannot be excluded. The constant appearance of congestion points to some inflammatory action. Certain experiments give further evidence

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of nephritic changes through a long continued albuminuria, through an acute globulinuria, etc.; but these changes are evidently not of such a degree as to give rise to marked degenerative lesions.

The intravenous injection of an extract of rabbit's *muscle* into two rabbits (experiments 68 and 69), showed very marked and extensive degeneration of the renal epithelium. The amount of urethane was very small in these cases (0.33 to 0.35 grm. per kilo.), so that the changes could not be attributed to it. Moreover, the other organs were normal. The degeneration was much more marked in the animal killed three days after injection, than in the one killed after 10 days. The kidneys showed no alterations in a case (experiment XLIV) of intravenous injection of dog's muscle and in experiment XXXIII of intravenous injection of human muscle into rabbits; nor in 2 rabbits (experiment XXXVIII), into whose peritoneum chicken's muscle was injected; nor in two others (experiment XXXIX), which received dog's muscle in the same manner. The degeneration of the renal epithelium, as noted in experiments 68 and 69, was therefore peculiar to this particular extract, and is not a common sequence of injections of muscle extracts.

The absence of conspicuous pathological lesions holds true still more for *other organs*. The spleen, intestines, pancreas and adrenals showed at most some congestion. The liver epithelium was very often greatly degenerated in rabbits which had received large doses of urethane; but this was plainly referable to the latter. Parasitic changes were often seen, and coccidium oviforme was very often found in the bile-ducts. In experiment 65 numbers of amœba-like parasites were seen in one kidney.

The absence of conspicuous degenerations is of interest not merely from its connection with the effects of proteid injection, but also because it bears on the much discussed question, whether uncomplicated hyperpyrexia causes cellular degeneration. We have not seen any such after elevations of temperature of one or two degrees lasting for two days. This tends to support Naunyn's view, corroborated lately by E. v. Czyhlarz (33), who saw no degenerations after cerebral puncture in ten rabbits.

Some attention was given to the distribution of pigment. Both hæmofuscin (iron free) and hæmosiderin (iron-containing) were found, in variable amount, in all cases. The latter was exclusively in the spleen. The former in the liver epithelium, in the spleen, both free and in cells, and often in the renal epithelium.

B.   FATALITIES OCCURRING IN THE COURSE OF THE EXPERIMENTS AND THEIR CAUSES.

Many of our animals succumbed in the course of the experiments. The greater number of these deaths could be referrd to well defined causes. Amongst these are embolism through two great concentration of the solution or from air; overdoses of the anæsthetic; bacterial infection; excessive handling or long exposure to unhygienic conditions, etc. A certain number pointed to a specific toxicity of the proteid. It seems to us of interest to give an illustrative description of the course and of the gross and microscopic changes in the principal fatal cases.

The phenomena of embolism are dealt with on page 241.

Deaths from the Anæsthetic.—Only one late death was observed in a dog (experiment XIX) which had received morphine and ether.

Experiment XIX.—This animal was very listless on the day following the operation; later it lay almost motionless in a comatose condition, and was found dead on the following morning. Autopsy negative.

The majority of deaths occurred in rabbits, after chloretone or urethane. The fatal dose for *chloretone* by stomach catheter, lies slightly above 16 cc. of a saturated solution per kilo. The symptoms were very similar to those produced by urethane. The degeneration of the liver cells was absent, nor were there any other histological lesions. There was, however, in all cases a marked capillary congestion of the abdominal organs.

The fatal dose of *urethane* given in 5% solution per rectum, lies between 0.75 to 1.0 grm. per kilo. But doses of 0.6 grm. cause very severe degeneration of the hepatic cells. Franz Müller (37), found that 1 grm. of urethane per kg. killed one rabbit out of three (in 2 days). When given by mouth the fatal dose of these anæthetics lies somewhat higher than by the rectum.

The following experiment forms a typical illustration:

Experiment XXXIII.—July 18, 1900. Rabbit ♂, 1790 grm., was anæsthetized with 1.5 grm. urethane, and ether; the jugular vein was exposed under aseptic precautions, and 125 cc. of a solution of human muscle in normal salt solution (= 0.486 grm. proteid) were injected.

The animal was partly conscious at the end of the operation (2.30 P. M.) and appeared in good condition. The wound was sutured and the rabbit placed in its cage. At 5.30 it was found lying on its side, very much worse. There had been, and was at this time, a profuse diarrhœa and

involuntary passages of a small quantity of urine. The heart was very fast; respiration 140; temperature 36.1° (normal rabbit = 40° C.); pupils of medium size, ear-vessels normal; reflexes slow. At 7 p. m. the respiration was 140; temperature 35.1°, heart fast, reflexes lost except corneal; pupil rather small. Heated bricks were placed about the animal at 7.15. At 7.25 the respiration became slowed to 12 and was coughing and spasmodic; temperature 34.4°. The animal died at 7.28, the respiration ceasing before the heart.

The *autopsy*, made immediately after death, showed a congestion of all abdominal organs. The small intestine was contracted and contained mucous fluid. The bladder was empty. The lungs were pink; the heart was not distended. The liver and kidneys presented a peculiar waxy appearance.

Histological Examination: (This refers to all the experiments in which urethane was used in a dose of 0.6 grm. per kg. or larger. With a dose of 0.35 grm. per kg. there were no noticeable lesions).

The principal changes are found in the *liver*. There is an intense diffuse degeneration of the hepatic epithelium, more marked in foci. The cells stain unevenly, are very granular, and present numerous vacuoles. Fatty degeneration is also sometimes seen. The nuclei are fairly normal. The perilobular tissue is normal. After large doses a congestion of the central vein and capillaries is also seen. The process of degeneration must be a very rapid one, for it is fully developed in animals which died 1½ hours after the injection (experiment XLVII). In the kidneys, some degree of congestion is common. The epithelium is always somewhat granular, but not more so than may be encountered in normal animals. The spleen is always congested. The other organs—intestine, medulla oblongata, pancreas, cardiac muscle, adrenals, are normal. The distribution of pigments shows nothing unusual.

Deaths from Bacterial Infection.—During May, 1901, an epizootic made its appearance amongst the guinea-pigs and rabbits at the laboratory, and carried off a great number of the former. The mortality was not so great with the rabbits, perhaps because they were not kept sufficiently long. With these animals, the disease was not suspected until the sections were examined. The disease occurred in animals which had not been handled, and was therefore not due to the injection.

The *microorganisms* as seen in the tissues were for the most part rather short pleomorphic bacilli, but larger numbers of cocci were often seen, especially in the lungs. Agar cultures were made from the guinea-pigs, but could not be examined for several weeks. They then showed a number of organisms, of which only B. coli communis could be identified. We are indebted to Mr. H. J. Gertenberger for these cultures.

The rabbits, even when intensely infected, showed but slight symptoms

during life. The guinea-pigs became listless on the last day, did not eat, lay on their side, and had a gasping respiration. The *autopsy* itself did not show any conspicuous changes. The numerous cases which were examined permit us to pursue the histological lesions through the successive stages of the infection. The bacteria were seen in all the tissues. The kidney showed some change in the epithelium of the convoluted tubules; the cells becoming somewhat swollen and cloudy or hyaline. There were no casts. The connective tissue was often fairly abundant. With more intense infection, the cell degeneration became very conspicuous. The connective tissue showed considerable round-celled infiltration. Gas-spaces were discernible. The liver cells showed at first a moderate, diffuse, granulation. In the perilobular tissue there was frequently more or less pronounced infiltration with young connective-tissue cells. The degeneration of the epithelium became very profound with the progress of the infection, being also somewhat focal. There were severe congestions and hæmorrhagic areas, the latter containing hæmosiderin. The spleen was congested, but otherwise normal even in the severe grades. The cardiac muscle was very profoundly affected, containing a mass of bacteria, and its structure having largely disappeared. The lungs were congested; the alveoli, bronchi, and blood contained many bacteria. The intestine, adrenals and pancreas were normal.

There was some hyperleucocytosis, and an increase of eosinophiles, especially in the spleen. Hæmosiderin and hæmofuscin were unusually abundant in the more marked grades of infection, especially the hæmosiderin in the spleen.

Toxic Effects of Egg-Albumen.—The following very striking instance of death following the intravenous injection of egg-albumen, which occurred rather early in the course of the research, directed our attention to the possibility of a toxic action of proteids which are usually considered harmless, and led us to an extensive experimental investigation of this subject.

Experiment XVIII.—This dog was but lightly anæsthetized during the operation. On the day following it was lively, ran about, and seemed perfectly well. It remained so during three days, and even at 8 A. M. on the 4th day nothing abnormal was noticed. The animal was not observed again until 1 P. M. of the 4th day, when she was found in extreme clonic spasms and almost insensible. Restorative measures were resorted to, but failed, death occurring at 1.30 P. M. The autopsy and histological study were both negative. The wound had suppurated a little.

This case is particularly interesting, being the only one in which an animal died rather suddenly after several intervening days of perfect good health. It seemed to us worth while to attempt to reproduce the condition, to enable us to study it more carefully.

As we had seen this course but once in more than 20 animals which we had injected, we searched for methods of increasing any existing toxicity of the egg-albumen. For this purpose we first tried the effect of repeating the injections on animals which are usually very sensitive to toxic agents.

We chose 6 guinea-pigs for the purpose, injecting them always in pairs, to exclude other accidental causes so far as possible. The injections were sometimes made under the skin, sometimes into the peritoneum. We were always successful in obtaining a fatal result, both animals of a pair always dying at the same time. Death usually occurred during the night succeeding the last injection, so that the final symptoms could not be observed. The guinea-pigs were always somewhat depressed after each injection, but nothing else could be noted. The autopsy showed nothing abnormal. There was no sign of peritonitis. However, the number of injections required, as well as the amount of proteid, were so large, that it seemed unjustifiable to refer the death to them. We were forced to conclude that the injection of fresh egg-albumen was in no way toxic.

Mariani (1897) has shown that starving rabbits treated with hypodermic injections of egg-albumen die before the control animals, but this might be referred to the increased nitrogen metabolism produced by such injections.

We may state at this place that the injections ordinarily produced few or no symptoms in either dogs or rabbits, except that the animals tended to become emaciated while preserving a ravenous appetite.

The phenomena of experiment XVIII must therefore have been due to the presence of some extraneous toxin in the egg-albumen. It remained to determine whether this was due to infection of the egg-solution, or whether such a toxin could be developed in the unspoiled egg itself. The eggs used for this experiment were purchased in July, in the open market, and were of unknown age. Whilst the eggs were not visibly spoiled, the conditions were distinctly favorable to fermentation. The cholin, etc., of the yolk would form a favorable starting point for such ptomains. To decide this question, we took some freshly laid eggs and placed them under conditions which would be most conducive to such change, *i. e.* in a thermostat

at 40° C. Here they were kept for 3, 5, 6 and 9 days. In the last case, the yolk could not be separated, and had to be included in the solution. Animals injected with solutions made from these brooded eggs behave precisely as if injected with normal egg-albumen. Only one case ended fatally, and this five days after the injection. The histological examination showed thrombosis of veins in the liver, perhaps quite sufficient to cause death; but we doubt whether this result could have been due to the injection.

We conclude that pure egg-albumen is not at all toxic, nor does it develop a toxicity by prolonged sojourn at brooding temperature.

This leaves bacterial infection as the explanation of experiment XVIII. Bacteriological examination was unfortunately neglected, but the tissues were certainly not conspicuously invaded by any micro-organism. The symptoms resembled superficially those of traumatic tetanus; since the wound was open, infection by this channel could have occurred quite easily. However, the sudden outbreak, the general distribution of the convulsions and rapid fatality speak very strongly against the existence of bacterial tetanus. The absence of any such effects in other animals operated upon, and kept under precisely the same conditions, makes it unlikely that the infection occurred by way of the wound. The toxic agent must have been introduced with the injected solution.

(Fiquet (34), 1899, claims that even albumoses and peptones are not toxic on intravenous injection, when prepared sufficiently pure, and that they then have no influence on the clotting of blood. He refers the ordinary action to admixture of toxalbumins and ptomains).

Toxic Effects of Alkali-Albumin.—Only one fatal case occurred in six intravenous injections of alkaline egg-syntonin in dogs, and two in rabbits. In this fatal case the alkalinity of the solution was unusually high, and the amount large (35 cc. per kilo. of a solution containing 0.376% of free NaOH). The same symptoms and lesions could be elicited in another animal by the injection of pure $\frac{N}{10}$ NaOH solution, so that they are not dependent upon the proteid. We reserve their detailed description for another paper.

Briefly, the animals died in about $6\frac{1}{2}$ hours, in coma and with convulsions. The autopsy revealed intense congestion of the abdominal

organs, particularly of the large intestine, with bloody effusions into the lumen of the alimentary canal and into the peritoneum.

Histologically, the main lesions were a necrosis of the intestinal villi and an acute interstitial nephritis, in every way similar to that described for the human subject by Councilman (35) and Howard (36). Hæmorrhagic areas were found in various situations. There was a profound hæmochromatosis, consisting mainly in the deposition of iron-free pigment granules in the intestinal mucosa, the liver, the blood, the lymph channels, and in the renal epithelium.

Other animals, which had received less alkali, showed much less marked changes in the same direction, or were free from lesions. Alkali-albumin, therefore, does not differ in its histological results from native albumen.

Muscle Extracts.—These did not possess any specific toxicity; the histological alterations corresponded to those seen after injection of egg-albumen.

VI.   CONCLUSIONS.

The conclusions which we derive from our observations are as follows:

1. The excretion of injected egg-albumen as such is in no case complete. The quantity retained varies from 23 to 100%.

2. The amount retained varies:

    a) directly with the slowness of absorption. This is determined by the manner of administration.

    b) directly with the time during which the proteid remains in the body; and therefore inversely to the rapidity of excretion.

    c) inversely to the quantity injected; this has however much less effect than (a) or (b).

    d) with individual peculiarities; but these are not very conspicuous.

3. The excreted proteid coagulates at the same temperatures as the injected albumen.

4. Injection of egg-albumen does not cause the appearance of globulins in the urine.

5. The proportion of proteid coagulating at lower temperatures is less in the urine than in the injected solution. When a solution has been heated to 73° before injection, the urine also does not coagulate below this temperature.

6. Egg-albumen injected into the hen is excreted as with mammals.

7. The albuminuria lasts in typical cases from $1\frac{1}{2}$ to 3 days, according to the manner of administration.

The excretion begins very shortly (7 minutes) after injection. 37 per cent of the total proteid injected may be excreted in an hour. About three-fourths of the total excretion takes place within the first 17 hours; the excretion is almost completed in the next 15 hours, only traces being excreted thereafter. With hypodermic injection the amount is more nearly equal on 2 or 3 successive days, since the absorption may extend over 2 days.

8. Alkali-albumin, as well as muscle-proteids (from foreign species) are completely retained. An unconverted mixture of egg-albumen and sodium carbonate behaves like egg-albumen.

9. A small amount of proteid (less than 5%) is excreted unchanged by the fæces.

10. A variable proportion is excreted as non-coagulable proteid. The quantity of this is proportional to that of the coagulable proteid of the urine.

11. The rest undergoes complete metabolism to urea.

12. The total nitrogen excretion is increased beyond the amount of nitrogen introduced as albumen.

13. Starvation appears to cause an increase in the ratio of the urea to the total nitrogen of the urine.

14. The effects of intravenous injection of egg-albumen on circulation and respiration do not differ from those of an equivalent injection of the solvent. Albumen causes, however, a specific diuresis, beginning 50 minutes after the intravenous injection, and reaching its maximum in about 2 hours. It causes neither glycosuria nor hæmoglobinuria.

15. The injection of egg-albumen, alkaline egg-syntonin, or muscle extracts, causes in rabbits a rise of temperature of 1 to 2° C.

This begins in about an hour, usually reaches its maximum in from 6 to 8 hours, and then falls rapidly. It may in rare cases persist for several days. It is indifferent qualitatively whether the injection is made by the jugular or the ear-vein, hypodermically, or into the peritoneum. Even extremely small quantities injected into the ear-vein cause this rise. The fever does not cause histological alterations in any organ examined. The injection of normal salt solution may cause a rise, but this is much smaller.

16. The injection of egg-albumen causes but very slight histological changes. The kidneys are usually congested, especially in the cortex. The cells may be slightly cloudy. A slight degree of nephritis may occur, but this is not of such degree as to effect permanent lesions. The injection of muscle extracts may give rise to a more pronounced parenchymatous nephritis.

17. Urethane is fatal to rabbits in doses of 0.75 to 1.0 grm. per kilo. The symptoms consist mainly in a very marked fall of temperature, and in medullary paralysis. 0.5 grm. per kilo. lowers the temperature 2.3° C. Doses as small as 0.6 grm. per kilo cause very marked histological changes, consisting mainly in extensive granular and vacuolar degenerations of the hepatic epithelium, which are so acute as to be fully developed when death occurs in 1½ hours after injection. Doses of 0.35 grm. per kilo. do not produce this change. Chloretone did not cause the degeneration, but is followed by congestion of the abdominal viscera.

18. Native egg-albumen, injected into the femoral vein of a dog, was followed in one case by a fatal ending with convulsions and coma, after several intervening cases of good health. Further experiments demonstrated that there is no toxicity inherent in fresh egg-albumen, nor can it be developed by brooding the eggs in the shell. The cause of the above fatal issue must therefore be sought in some extraneous toxic agent which contaminated the solution. Muscle-extracts were also devoid of toxicity. Alkali-albumin produces no changes beyond those which may be attributed to the free alkali contained therein.

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