CCXXIV. THE EFFECTS OF INSULIN AND ADRENALINE ON THE AMINO-ACID CONTENT OF BLOOD.

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In previous work it has been shown that insulin, injected into the normal animal, reduces the amino-nitrogen content of the blood and tissues [Luck *et al.*, 1928; Kiech and Luck, 1928], the latter even though hypoglycaemia be prevented by the simultaneous administration of glucose [Luck and Spaulding, 1928–29]. The rate of urea formation is also increased. More and more we have come to believe that these effects upon protein metabolism are independent of the effects upon carbohydrate metabolism. In the present paper we wish to adduce additional evidence which leads to this conclusion.

Experiments with crystalline insulin.

First of all it became necessary to determine for a certainty whether the amino-acid-lowering activity of insulin, hitherto studied upon therapeutic preparations only, was possessed by the crystalline hormone. We were aware of the possibility of the amino-acid activity being due to associated impurities, in much the same way that the transitory hyperglycaemic action of amorphous insulin is probably due to some principle other than insulin itself [Bürger and Kramer, 1930; Geiling and de Lawder, 1930]. Professor du Vigneaud was good enough to send us a specimen of crystalline insulin with which we sought an answer to the question.

Seven rabbits, previously starved for 48 hours, were injected subcutaneously with a freshly-prepared solution of crystalline insulin (1 cc. per kg. of body weight of a solution containing 1 unit per cc.). Two blood samples of 2 cc. were drawn from each—the first a few minutes before injection, the second 3½ hours after[1]. Reducing sugar and amino-acid nitrogen were determined by the methods of Folin [1922; 1928; 1929]. The following values were obtained.

									Mean
Amino-acid-N mg. per 100 cc.	Before	8·2	8·2	8·6	8·1	9·0	9·1	8·9	8·6
	After	6·7	6·9	6·6	6·5	7·4	7·8	7·4	7·0
Sugar mg. per 100 cc.	Before	99	96	97	94	92	94	94	95
	After	59	38	44	47	59	58	58	52

[1] To avoid hypoamino-acidaemia due to haemorrhage only two blood samples were drawn. Five animals receiving injections of saline in place of insulin and bled at 0, ¾, 1½, 2½, 3½ and 4½ hours after injection showed decreases in blood-amino-nitrogen content of 1·3 mg./100 cc. (mean value). Seven other rabbits which were bled but twice (0 and 3½ hours after injection) showed no decrease. In two insulinised rabbits with six bleedings decreases of 3·2 mg./100 cc. were observed. The effect was maximum after 3½ hours. These results are sufficient to account for our adoption of the "two-sample" procedure.

In the next paper of this series many more experiments, some with a different preparation of crystalline insulin, will be reported. The results are identical with these. It is apparent that the hypoamino-acidaemia produced by ordinary insulin is a result of the hormone itself, not of impurities.

Prevention of hypoglycaemia by the simultaneous injection of adrenaline. It occurred to us that a simple method of preventing hypoglycaemia arising from insulin administrations would be by simultaneous injections of adrenaline. An opportunity would thereby be presented of studying the rôle of hypoglycaemia in insulin hypoamino-acidaemia.

In the first group of experiments rats were employed. Attention was given to the effect of balanced injections of insulin and adrenaline upon the amino-nitrogen content of liver and muscle. By way of introduction it should be mentioned that earlier experiments demonstrated to us that the lowering of the amino-nitrogen content of blood induced by insulin is accompanied by a simultaneous reduction in the amino-nitrogen content of liver and muscle. This is in harmony with the finding of Kiech and Luck [1928] that the amino-nitrogen content of the animal as a whole suffers reduction.

In the present experiments 72 animals, starved for 36–40 hours at 28°, were used. The optimum conditions for obtaining a balancing of insulin hypoglycaemia by means of adrenaline were first determined. Samples were collected from the tip of the tail at −15, 5, 30, 60, 90 and 120 minutes after the initial injection. By repeated trial it was found that satisfactory control of the hypoglycaemia was obtained by injecting simultaneously 5 units of insulin per kg. of body weight, and 0·38 mg. of adrenaline per kg. followed by a second dose of adrenaline 65 minutes later (0·250 mg. per kg.).

Blood-sugar content (mg./100 cc.).

Animal	−15 min.	5 mins.	30 mins.	60 mins.	90 mins.	120 mins.
21	91	99	121	112	106	96
22	86	106	119	124	115	119
23	101	109	134	112	108	102
24	96	129	166	143	123	102
25	—	88	118	116	108	92

In three other animals from each of which only one sample was collected (2 hours after the initial injection) blood-sugar values of 94, 111 and 82 mg./100 cc. were obtained. Three other animals, paired with these three, were similarly treated except that 0·9 % saline was injected instead of the adrenaline. Values of 40, 43 and 39 mg./100 cc. were obtained.

Amino-nitrogen determinations on liver and muscle. The animals, injected both with insulin and adrenaline as described, were killed 2 hours after the first injection. The liver and thigh muscles were promptly excised, frozen in liquid air, powdered and analysed for amino-nitrogen [Luck, 1928]. The mean values presented in Table I were obtained.

Table I. *Amino-nitrogen content of liver and muscle.*

	Liver		Muscle	
	Number of animals	Amino-N content per 100 g. liver mg.	Number of animals	Amino-N content per 100 g. muscle mg.
Saline controls	23	47·7	24	58·8
Insulin	15	44·9	19	54·7
Adrenaline	11	44·6	10	54·9
Insulin *plus* adrenaline	15	43·3	18	55·9

It follows that the reductions obtained in the amino-nitrogen content of liver and muscle by injection of insulin are not appreciably lessened, if at all, by the simultaneous injection of adrenaline. In fact, adrenaline alone was found to lower the amino-nitrogen content—a point of some importance to which we shall presently return.

The rats used in the experiments just described were analysed in pairs, so selected that the members of any given pair were of the same sex, closely similar in age and weight, and, when possible, of the same litter. The one member of the pair received either saline or insulin, while the other received either adrenaline or insulin *plus* adrenaline. Analysis of the data from such "paired" experiments led to the same conclusion. Thus we observed in muscle that although the amino-nitrogen content of the groups of saline controls varied from 57·6 to 60·2, the values obtained from the experimental animals paired with these were uniformly less. For example, six rats which received insulin had an average amino-nitrogen content in muscle of 53·0. The saline controls paired with these averaged 57·6. The decrease induced by insulin was 4·6 mg./100 g. The corresponding values for nine adrenaline animals and their saline controls were 54·7 and 58·2; net decrease 3·5. For six animals receiving insulin *plus* adrenaline the corresponding values were 56·8, 60·2 and 3·4.

The changes in the basal level of amino-nitrogen content, reflected in the shifting saline-control values, seem to be partly seasonal in nature. They are of greater magnitude in liver but may be satisfactorily controlled by adoption of the paired-animal method.

To obtain confirmatory data we approached the problem afresh by studying the effect of balanced injections of insulin and adrenaline on the amino-nitrogen content of the blood of rabbits. The animals were bled but once, 3½ hours after the initial injection. The most satisfactory control of the hypoglycaemia was obtained by injecting simultaneously 1 unit of insulin per kg. of body weight and 0·075 mg. of adrenaline per kg. followed by two injections of adrenaline (0·05 mg. per kg.) after 65 minutes and 2 hours respectively. In two animals,

Table II. *The effect of balanced injections of insulin and adrenaline on the sugar and amino-nitrogen content of blood.*

	Sugar mg. per 100 cc. blood		Amino-N mg. per 100 cc. blood	
Animal	0	3½ hours	0	3½ hours
1 A	105	88	9·4	7·8
2 A	100	105	9·3	7·6
1 B	112	118	7·1	6·7
2 B	106	115	9·6	7·3
3 B	108	83	8·3	8·2
5 B	121	129	8·8	8·7
6 B	100	149	8·8	8·1
7 B	110	114	8·9	8·2
5 B	115	118	8·8	7·5
7 B	88	119	8·1	6·5
2 C	114	82	10·0	7·3
1 C	119	91	9·3	7·0
3 B	121	110	7·8	6·9
6 B	119	149	8·4	6·6
7 B	105	118	8·5	6·6
3 C	118	108	10·2	8·2
4 C	122	128	9·3	6·6
5 C	100	95	8·3	6·5
	110	112	8·8	7·3

so injected, five blood-sugar determinations were made during 3½ hours following the initial injection. All the values fell between 99 and 118 mg./100 cc. Eighteen experiments were performed. Despite the absence of hypoglycaemia a definite fall in amino-nitrogen was observed, the decrease being 17 % as against a mean fall of 21 % resulting from insulin alone. The means for all experiments follow:

Amino-N	Before	8·8	Sugar	Before	110·2
mg. per 100 cc.	After	7·3	mg. per 100 cc.	After	112·2

The complete results are presented in Table II. They indicate that a persistent hypoamino-acidaemia is obtained even when hypoglycaemia has been prevented.

Experiments with adrenaline alone.

Although the literature contains two papers [Loeper *et al.*, 1926; Putschkow and Krassnow, 1928] on the amino-nitrogen content of the blood of adrenalectomised animals and other communications on the effect of adrenaline upon various aspects of protein metabolism, the results reported are markedly discordant. Since no one seems to have studied the effect of adrenaline upon the amino-nitrogen content of the blood of normal animals, we undertook the experiments summarised in Table III. Fasting rabbits again were used. It will be seen that adrenaline is fully as effective as insulin in lowering the amino-nitrogen content of blood.

Table III. *The effect of adrenaline on the amino-nitrogen content of blood.*

	Sugar mg. per 100 cc. blood		Amino-N mg. per 100 cc. blood	
Animal	0	3½ hours	0	3½ hours
1 A	116	238	10·1	8·5
2 A	114	263	9·8	7·5
3 B	104	256	8·6	7·7
2 B	102	250	9·3	7·2
5 B	111	298	9·2	7·8
7 B	110	282	9·6	7·5
6 B	—	254	9·0	7·0
8 B	111	200	8·6	6·8
	110	255	9·3	7·5

Experiments with posterior pituitary extracts and insulin.

The known antagonism between certain pituitary principles and insulin [Burn, 1923; Olmsted and Logan, 1923] led us to investigate the effect of posterior pituitary extracts upon insulin hypoamino-acidaemia.

In the first group of experiments extracts of Armour's powdered posterior pituitary were used. The extracts were prepared according to the U.S. Pharmacopoeia [1926] and were based on the acetic acid extraction method of Aldrich [1908]. In the remaining experiments the oxytocic and vasopressor preparations of Kamm *et al* [1928] known as pitocin and pitressin were used[1]. Fasting rabbits were employed throughout. The results, presented in Table IV, show that pitocin with insulin caused very substantial decreases in the amino-nitrogen content of blood, the decreases being somewhat greater than we generally observe with insulin alone. At the same time the hypoglycaemic action of the insulin was reduced, especially with the smaller dose of pitocin (12 units/kg.).

[1] We are indebted to Parke, Davis and Company for supplying the pitocin and pitressin used in this work.

Table IV. *The effect of posterior pituitary extracts and insulin on the sugar and amino-nitrogen contents of blood.*

Sugar mg. per 100 cc. blood				Amino-N mg. per 100 cc. blood				Preparation used in conjunction with insulin
0	1 hour	2½ hours	3½ hours	0	1 hour	2½ hours	3½ hours	(1 unit/kg.)
117	75	49	52	8·6	8·3	8·3	8·2	Acetic acid extract 1 cc./kg.
98	73	51	56	9·7	9·4	8·5	9·0	
106	92	70	71	9·3	—	7·2	6·9	Acetic acid extract 2 cc./kg.
115	67	61	69	7·8	8·1	6·7	6·7	
125	112	89	99	8·1	7·2	6·4	6·2	Pitocin 12 units/kg.
101	133	95	80	9·2	8·8	7·3	7·0	,, ,,
113	73	68	60	10·8	9·5	8·2	7·9	Pitocin 18 units/kg. (divided dose)
122	70	63	66	10·0	8·6	8·0	7·9	,, ,,
110	80	67	69	8·0	8·7	6·8	6·8	Pitressin 10 units/kg.
100	117	—	65	8·1	8·5	6·8	6·8	Pitressin 15 units/kg.
114	74	71	78	9·0	10·0	8·4	7·9	Pitressin 15 units/kg. (divided dose)
93	83	69	57	8·9	9·6	7·8	7·2	Pitressin 20 units/kg. (divided dose)
110	118	69	65	9·1	9·6	8·1	7·9	,, ,,
114	123	61	71	8·8	9·1	8·8	8·5	,, ,,

Pitressin given with insulin reduced somewhat the hypoglycaemic action of the latter with little if any effect upon its amino-nitrogen-lowering activity, with the curious exception, possibly, of an early but transitory increase in the amino-nitrogen content [*cf.* Bischoff and Long, 1931]. The anhydraemia usually caused by pitressin may account, partially, for this increase in amino-nitrogen. In one animal hypoamino-acidaemia was not observed.

The results with the acetic acid extracts are not easily interpreted. The larger dose of extract decreased somewhat the hypoglycaemic effect of insulin without altering its ability to lower amino-nitrogen. The smaller dose was without effect in lessening the hypoglycaemia but, oddly enough, was accompanied by but little hypoamino-acidaemia.

It should be mentioned that controls with insulin, pitressin and acetic acid extracts alone were run. Pitressin alone caused a transitory increase in amino-nitrogen similar to that obtained with insulin and pitressin together.

In harmony with the adrenaline experiments the results indicate that the amino-nitrogen-lowering activity of insulin is independent of its hypoglycaemic activity. However, the neutralisation of the hypoglycaemia by the pituitary preparations was complete in only two cases, so the results, though supporting the conclusions drawn from the adrenaline experiments, do not constitute a complete proof in themselves.

Incidentally the data on blood-sugar lend support to the conclusion of Magenta [1929] that pitocin is more active than pitressin as an antagonist to insulin.

Discussion.

The principal result of these studies to which we wish to draw attention is the independence of the hypoamino-acidaemia induced by insulin of the characteristic effect upon carbohydrate metabolism—hypoglycaemia. An explanation, we feel, may be found in the experiments with adrenaline alone which caused decreases in amino-nitrogen content fully as great as those obtained with insulin alone. The fact that injected insulin provokes an increased secretion of adrenaline [Cannon *et al.*, 1924; Houssay *et al.*, 1924; Kugelmann, 1931; La Barre and Houssa, 1932] leads us to suggest, in the light of our present findings, that adrenaline rather than insulin may be responsible for the decreases in amino-nitrogen reported in this series of papers. Other results obtained by the use of partially inactive insulin [Davis *et al.*, 1933] and low dosages of active insulin [Luck and Van Winkle] strongly support this conclusion. If adrenaline be the agent responsible for hypoamino-acidaemia, it is to be expected, as we have shown, that the prevention of insulin hypoglycaemia by adrenaline would be without effect upon the amino-nitrogen reduction. We are unaware of any work which indicates whether insulin would continue to provoke an increased secretion of adrenaline when hypoglycaemia is completely prevented by the simultaneous administration of glucose. Should it not do so, the observations of Luck and Spaulding [1928–29] could not be reconciled with the hypothesis that adrenaline, to the exclusion of insulin, is responsible for the hypoamino-acidaemia reported in this series of experiments. In consequence, the possibility that amino-acid lowering may be a primary and independent function of both insulin and adrenaline cannot be ignored. We hope that other experiments, to be undertaken before long, may give a conclusive answer to this question.

SUMMARY.

1. Crystalline insulin is as effective as amorphous insulin in reducing the amino-nitrogen content of the blood of normal rabbits.

2. The amino-nitrogen contents of liver and muscle are reduced alike by insulin, adrenaline, or balanced injections of insulin and adrenaline together.

3. Though hypoglycaemia be prevented by the use of balanced injections of insulin and adrenaline, hypoamino-acidaemia is still observed.

4. Adrenaline alone is as effective as insulin in reducing the concentration of blood-amino-nitrogen.

5. Pitocin lessens the hypoglycaemic action of insulin and increases slightly the amino-acid-lowering activity.

6. Pitressin given with insulin causes a slight and transitory hyperamino-acidaemia followed by hypoamino-acidaemia.

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