REFERENCES

Birkinshaw, J. H. & Raistrick, H. (1931). *Philos. Trans.* B, **220**, 245.
Birkinshaw, J. H. & Raistrick, H. (1936). *Biochem. J.* **30**, 2194.
Clift, F. P. & Cook, R. P. (1932). *Biochem. J.* **26**, 1802.
Clutterbuck, P. W., Haworth, W. N., Raistrick, H., Smith, G. & Stacey, M. (1934). *Biochem. J.* **28**, 94.
Clutterbuck, P. W., Lovell, R. & Raistrick, H. (1932). *Biochem. J.* **26**, 1907.
Clutterbuck, P. W., Raistrick, H. & Reuter, F. (1935*a*). *Biochem. J.* **29**, 300.
Clutterbuck, P. W., Raistrick, H. & Reuter, F. (1935*b*). *Biochem. J.* **29**, 871.
Clutterbuck, P. W., Raistrick, H. & Reuter, F. (1935*c*). *Biochem. J.* **29**, 1300.
Johnson, J. R., Bruce, W. F. & Dutcher, J. D. (1943). *J. Amer. chem. Soc.* **65**, 2005.
Johnson, J. R., McCrone, W. C. & Bruce, W. F. (1944). *J. Amer. chem. Soc.* **66**, 501.
Menzel, A. E. O., Wintersteiner, O. & Hoogerheide, J. C. (1944). *J. biol. Chem.* **152**, 419.
Oxford, A. E. & Raistrick, H. (1942). *Chem. Ind.* **61**, 128.
Reeves, R. E. (1941). *J. Amer. chem. Soc.* **63**, 1476.
Weindling, R. & Emerson, O. H. (1936). *Phytopathology*, **26**, 1068.

The Action of Vitamin K in Hypervitaminosis A

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Moore & Wang (1943, 1945) have given a comprehensive review of the toxic effects produced in experimental animals by greatly excessive amounts of vitamin A. From their experiments with pure vitamin A acetate they concluded that the most characteristic lesions were skeletal fractures and haemorrhage, the first of these lesions being predominant in growing animals and the second in adult animals. The fractures caused by pure vitamin A have been confirmed by Herbst, Pavcek & Elvehjem (1944) and Pavcek, Herbst & Elvehjem (1945) who also found that telangiectatic bovine livers, rich in vitamin A, are toxic to rats. Light, Alscher & Frey (1944) approached the problem from a new angle, by showing that hypervitaminosis A in rats was associated with a pronounced hypoprothrombinaemia, which could be corrected by giving vitamin K as 2-methyl-3-phytyl-1:4-napthaquinone. The object of the present investigation has been to confirm and extend this important observation.

EXPERIMENTAL

Method

In a series of experiments, piebald rats were fed on massive doses of vitamin A for periods varying from 10 to 18 days, with or without the addition of vitamin K, or, in one experiment, of vitamin C. The source of the vitamin A was halibut-liver oil, containing 40,000 i.u./g., which was kindly supplied by Allen and Hanbury, Ltd. At the termination of each experiment the rats were killed under ether anaesthesia. The chest wall was opened and as much blood as possible withdrawn from the heart into an oxalated syringe. The livers were removed, weighed, and placed in 5% KOH solution, and the bodies were X-rayed for the presence of fractures. Vitamin A was estimated in the livers by the antimony trichloride method applied after the digestion of the tissues by alkali (Davies, 1933) and in the blood by the same method applied according to the technique of Yudkin (1941). Plasma clotting times were measured by the method of Campbell, Smith, Roberts & Link (1941) and an approximate measurement was made of the plasma/cell ratio by centrifuging the blood for a standard time in graduated centrifuge tubes. Unfortunately no haematocrit was available.

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Exp. 1–4. Piebald rats of both sexes, usually young but in one experiment nearing maturity, were given a basal diet of casein 20%, sugar 60%, fat 15% and salt mixture 5%, with dried yeast 10% (additional). Vitamin A was supplied as one drop of halibut-liver oil/rat/week, vitamin D as one drop of 'Radiostol', and vitamin E as 1 mg. of *dl*-α-tocopheryl acetate. Each experiment, except no. 4, included three groups which are described in Tables 1 and 2 as 'control', 'excess of vitamin A' and 'excess of vitamin A + vitamin K'. In the control groups the fatty component of the basal diet was supplied as arachis oil, whereas groups given excess of vitamin A received their fat as halibut-liver oil, which was freshly mixed each day with the solid components of the diet. In Exp. 1 the group given vitamin K in addition to vitamin A received it in the form of 'Synkavit' (tetrasodium 2-methyl-1:4-naphthahydroquinone diphosphoric acid ester). The Synkavit was added to the dry constituents of the diet so as to supply about 160 μg./rat daily. To avoid any possible criticism that the admixture

with the diet was uneven, however, vitamin K was given in subsequent experiments as 80 μg. of 2-methyl-1:4-naphthaquinone three times weekly, the dose being administered in oily solution to each animal by dropping pipette.

Since the lesions caused by excess of vitamin A vary with the age of the animal the sizes of the animals used in the various experiments are relevant. The rats used in Exps. 1 and 2 had initial body weights of 43–85 g., and may be described as 'young' rats. The rats used in Exp. 3 had weights of 125–153 g., and may be described as 'adolescent'. The rats used in Exp. 4 were 'young' rats of weights 83–126 g. Exps. 2 and 3 were carried out simultaneously, and the results for the young and adolescent rats in these experiments are therefore strictly comparable. In Exps. 1–3 the animals were killed without previous fasting, but in Exp. 4 (Table 3) the animals were fasted for 24 hr. before being killed.

RESULTS

As already stated the most characteristic lesions in hypervitaminosis A appear to be haemorrhages, variable in intensity and distribution, and also spontaneous skeletal fractures, which occur mainly in young rats. Both these injuries were produced in various groups in the present experiments, and the apparent effect of age was confirmed. Thus at autopsy the haemorrhages found in adolescent animals of Exp. 3 were more widespread and severe than those in young rats, but spontaneous skeletal fractures were absent. The only evidence of softness in the bones in the adult groups was that in two instances fractures were produced accidentally while

Table 1. *Effect of vitamin K on hypervitaminosis A in young rats*

		Rat			Vitamin A: Liver		Vitamin A: Blood concentration (i.u./100 ml.)	Plasma prothrombin time (sec.)	Plasma/Cell ratio	Haemorrhages†	Fractures†
	Exp.	No.	Wt.* (g.)	Days on exp.	Concentration (i.u./g.)	Total (i.u.)					
1.	Controls	1	72	12	144	760	74	31	1·2	0	0
		2	65	18	145	530	67	31	1·2	0	0
				Means	**144**	**650**	**71**	**31**	**1·2**		
1.	Excess of vit. A	3	80	18	9,200	42,000	240	100	2·1	+	0
		4	66	12	11,100	49,000	890	29	3·0	0	+ + +
		5	70	18	4,400	18,000	250	35	2·6	+	+ +
		6	82	18	5,300	24,000	290	143	2·7	0	+ +
		7	66	18	13,500	49,000	240	45	2·0	+	+ + +
				Means	**8,700**	**36,000**	**364**	**70**	**2·5**		
1.	Excess of vit. A + vit. K	8	75	18	3,600	10,800	2,050	32	2·2	+ +	+
		9	64	12	8,100	26,000	4,960	29	2·0	+	+ + +
		10	79	18	7,100	27,000	130	32	1·8	0	+ + + +
		11	79	18	4,500	18,000	210	30	2·3	0	+
		12	81	12	7,100	25,000	1,330	29	2·0	0	+ +
				Means	**6,100**	**21,000**	**1,740**	**30**	**2·1**		
2.	Controls	13	51	17	300	1,410	84	38	1·5	0	0
		14	43	17	140	680	90	41	0·9	0	0
		15	50	10	94	260	30	C	—	0	0
		16	49	10	186	520	50	28	1·4	0	0
				Means	**180**	**720**	**64**	**36**	**1·3**		
2.	Excess of vit. A	17	85	17	1,500	8,400	400	101	3	+	+ + +
		18	70	10	4,800	21,000	470	81	2	+	+ +
		19	70	10	2,600	15,200	1,330	68	2	0	+
		20	70	17	9,500	46,000	350	354	2·2	0	+ +
		21	69	10	8,800	37,000	140	60	1·6	0	+ +
		22	78	17	12,700	62,000	640	51	2·2	0	+ + +
				Means	**6,700**	**32,000**	**555**	**119**	**2·2**		
2.	Excess of vit. A + vit. K	23	51	12D	4,600	13,900	—	—	—	0	+ + +
		24	76	17	8,800	35,000	1,550	32	1·5	+	+ + + +
		25	77	10	6,000	26,000	300	25	1·3	0	+ + +
		26	66	10	7,100	33,000	1,760	24	1·6	0	+ + +
		27	64	17	4,700	21,000	1,010	31	2·3	+	+ + +
		28	64	10	4,300	17,500	440	27	1·7	0	+ +
				Means	**5,900**	**24,400**	**1,010**	**28**	**1·7**		

* Initial weight.
† The number of crosses indicates the general intensity of haemorrhage or the number of fractures.
C = clotted on withdrawal. D = died.

Table 2. *Effect of vitamin K on hypervitaminosis A in adolescent rats*

Exp.		Rat No.	Rat Wt.* (g.)	Rat Days on exp.	Vitamin A: Liver Concentration (i.u./g.)	Vitamin A: Liver Total (i.u.)	Vitamin A: Blood concentration (i.u./100 ml.)	Plasma prothrombin time (sec.)	Plasma/Cell ratio	Haemorrhages†	Fractures†
3.	Controls	29	193	10	25	205	69	27	0·9	0	0
		30	165	10	28	196	50	32	0·8	0	0
		31	200	17	56	515	100	44	0·8	0	0
		32	196	17	98	1,010	100	34	1·3	0	0
				Means	**52**	**482**	**80**	**34**	**1·0**		
3.	Excess of vit. A	33	190	9	5,600	50,000	290	202	2·8	+ + +	0
		34	173	10	5,900	48,000	80	226	3	+	0
		35	168	17D	7,200	88,000	—	—	—	+ + +	+ +‡
		36	190	10	5,200	41,000	60	89	2·2	+ + +	0
		37	151	17	12,300	96,000	380	101	1·1	0	0
		38	144	17	11,400	92,000	220	281	1·3	+	0
				Means	**7,900**	**69,000**	**206**	**180**	**2·1**		
3.	Excess of vit. A + vit. K	39	127	10	8,200	56,000	210	27	1·3	0	0
		40	140	17	11,800	87,000	310	33	1·2	0	0
		41	153	17	14,100	97,000	700	30	1·4	0	0
		42	133	10	8,000	55,000	150	27	1·2	0	0
		43	143	10	7,600	56,000	130	28	0·9	0	0
		44	125	17	11,300	74,000	270	30	1·6	0	+‡
				Means	**10,200**	**71,000**	**295**	**29**	**1·3**		

* Initial weight.
† The number of crosses indicates the general intensity of haemorrhage or the number of fractures.
‡ Sustained during handling post-mortem. D = died.

Table 3. *Effect of vitamin K and of vitamin C on hypervitaminosis A in young rats fasted for 24 hr. before being killed*

Exp.		Rat No.	Rat Wt.* (g.)	Rat Days on exp.	Vitamin A: Liver Concentration (i.u./g.)	Vitamin A: Liver Total (i.u.)	Vitamin A: Blood concentration (i.u./100 ml.)	Plasma prothrombin time (sec.)	Plasma/Cell ratio	Haemorrhages†	Fractures†
4.	Excess of vit. A	45	131	18	22,000	54,000	145	68	—	+	0
		46	93	18	27,000	117,000	126	37	—	0	+ +
		47	95	18	15,800	48,000	133	76	—	+	+ +
		48	110	18	23,000	70,000	370	52	—	+	+
		49	98	18	18,800	66,000	242	—	—	+	+ +
		50	103	18	8,400	42,000	144	90	—	0	+ +
				Means	**19,200**	**66,000**	**193**	**65**			
4.	Excess of vit. A + vit. K	51	91	18	9,400	42,000	96	35	—	0	+ +
		52	86	18	12,700	48,000	152	37	—	0	+ +
		53	126	18	14,500	86,000	104	32	—	0	+ +
		54	92	18	12,800	50,000	363	—	—	0	+ +
		55	83	18	27,000	68,000	160	—	—	0	+ +
		56	89	18	9,300	28,000	211	38	—	0	+ + +
		57	88	18	10,700	39,000	144	30	—	0	+ +
				Means	**13,800**	**52,000**	**176**	**34**			
5.	Excess of vit. A + vit. C	58	88	18	23,200	70,000	142	—	—	+	+ +
		59	106	18	9,900	44,000	122	46	—	+	+ +
		60	92	18	10,200	41,000	167	42	—	0	+ +
		61	95	18	12,600	44,000	542	68	—	+	0
		62	94	18	14,900	62,000	120	—	—	0	+ +
		63	85	18	10,300	36,000	139	45	—	0	+ +
		64	86	18	14,400	53,000	291	47	—	+	+ +
				Means	**13,600**	**50,000**	**218**	**50**			

* Initial weight.
† The number of crosses indicates the general intensity of haemorrhage or the number of fractures.

the animals were being handled after death. During the course of the experiments the older animals seemed to be in a poorer state of health than the young ones, and their food consumption, and hence their intake of vitamin A, was smaller. This may account in part for the difference in the reactions of the younger and older rats to excess of vitamin A.

The recent claim that hypervitaminosis A is accompanied by hypoprothrombinaemia, as indicated by a prolonged plasma clotting time, was confirmed in all groups given halibut-liver oil without vitamin K. The administration of vitamin K either as 2-methyl-1:4-naphthaquinone or as 'Synkavit' prevented this abnormality. The effect of vitamin K on haemorrhage was somewhat less clear cut. In adolescent rats which were given vitamin K (Exp. 3) no haemorrhages were found, but the results were less definite in the younger animals. On the other hand vitamin K had no effect on the skeletal lesions. Spontaneous fractures occurred in all young rats given excess of halibut liver oil, and accidental fractures after death in one animal in each of the groups of adult rats, irrespective of whether vitamin K was given or not.

Our original purpose in estimating vitamin A in the livers and tissues was to obtain information as to the levels which were associated with the development of toxic symptoms. The results for Exps. 1–3, in which the rats were killed without preparatory fasting, indicate the prevailing levels at the conclusion of the experiment, as influenced by the usual habits of the animals in ingesting their food. The values for liver in rats receiving excess of vitamin A were of the same order as those found in hypervitaminotic rats by Davies & Moore (1934). The values for blood plasma were much higher than in control rats. The decision to fast the animals in Exp. 4 before killing was taken since it appeared from the results of Exps. 1 and 2 that vitamin K might possibly have the effect of raising the level of vitamin A in the plasma and reducing the amount stored in the liver. In attempting to confirm this effect it seemed desirable to eliminate the possibility of large temporary variations in the plasma vitamin A, which might be expected from experience with other animals to occur within the first few hours after ingesting a massive dose. After this precaution the mean vitamin A contents of the plasma of animals treated or not treated with vitamin K were found to be virtually identical, while the difference between the mean liver reserves was small in relation to the wide variations which occurred within each group.

Another effect which has not been reported previously was found in rats given excess of vitamin A. On withdrawal their blood appeared to be thin and watery. The mean plasma/cell ratio for all animals given excess of vitamin A without vitamin K was 2·2, as compared with 1·2 for control animals. When vitamin K was given in addition to vitamin A the mean ratio was 1·6.

The action of vitamin C in hypervitaminosis A

Vedder & Rosenberg (1938) claimed that ascorbic acid is beneficial to rats given excess of jew-fish-liver oil, a rich source of vitamin A. Moore & Wang (1945), however, failed to improve the condition of rats receiving excess of vitamin A acetate by giving them large doses of ascorbic acid. In the present investigation it seemed of interest to re-examine this point, with particular reference to the effect of ascorbic acid on the prothrombin time.

Exp. 5. This experiment was carried out simultaneously and in parallel with Exp. 4. A group of rats, receiving excess of halibut-liver oil, was dosed with 50 mg. of ascorbic acid/rat daily by dropping pipette. Table 3 indicates that the ascorbic acid gave no protection against either haemorrhages or fractures. The mean prothrombin time was less than in the untreated group, but greater than in the group dosed with vitamin K.

Dicumarol poisoning

Further evidence that skeletal fractures and hypoprothrombinaemia may be produced separately was obtained in experiments with dicumarol. This substance is known to produce a marked fall in blood prothrombin levels in various species (Overman, Stahmann, Sullivan, Huebner, Campbell & Link, 1942). Therefore it was considered desirable to investigate whether the hypoprothrombinaemia of dicumarol poisoning was accompanied by any bony changes which might produce fractures.

Exp. 6. Twelve young rats of weights ranging from 36 to 55 g. were given daily doses of dicumarol varying from 1 to 3 mg. Their diet was identical with that of the controls in the experiments described above, the dicumarol being added to the food. The animals died within 3–7 days and at autopsy severe and extensive haemorrhages were found. Careful examination was made of the limbs but no fractures or visible bone abnormalities were seen. As the survival time was so short the experiment was repeated, with smaller doses calculated on a body-weight basis and slightly older rats (80–97 g.) in order to observe the effect of a more prolonged action of dicumarol. The doses in this instance were from 1·5 to 5 mg., and the survival times 10–38 days. The post-mortem findings were similar to those in the preceding experiment, and again no fractures were found.

DISCUSSION

In confirmation of the results of Light, Alscher & Frey (1944) we have found that an injurious excess of vitamin A causes prolongation of the plasma

clotting time in rats, which is presumed to indicate a condition of hypoprothrombinaemia. This abnormality could be corrected by the administration of vitamin K. It would appear therefore that toxic excess of vitamin A induces a secondary deficiency of vitamin K. There is a variety of ways in which this could arise. Since, on ordinary experimental diets, the rat can exist without any extraneous source of vitamin K it might be considered that the excess of vitamin A interferes with the synthesis of vitamin K by the intestinal flora. Another possibility is that vitamin A in toxic doses may interfere with the absorption either of small amounts of vitamin K which may be present in some unsuspected source in the basal diet, or of the vitamin K which has been synthesized in the gut. Yet another mechanism might be the destruction or inactivation of the vitamin K within the liver or elsewhere in the body. Finally, it is conceivable that excessive amounts of vitamin A or its metabolites might in some way increase the rat's demand for vitamin K.

Although rats given vitamin K invariably had normal prothrombin times, the action of vitamin K in preventing haemorrhage was consistent only in rats which had initial body weights of about 85 g. and over. Thus out of 6 rats in Exp. 3 and 7 rats in Exp. 4 which were dosed with vitamin K, none had haemorrhage. Among the smaller rats used in Exps. 1 and 2, however, 4 which were dosed with vitamin K had haemorrhage. Moreover, two of those not dosed with vitamin K which had haemorrhage gave normal prothrombin times.

In agreement with previous work (Moore & Wang, 1945) excess of vitamin A also caused spontaneous fractures of the bones in young, but not in adolescent, rats. It may be noted that the division according to body weight which determined the incidence of fractures was not the same as that which was found for the action of vitamin K in preventing haemorrhage. Thus the rats in Exp. 4 which sustained fractures were nevertheless protected against haemorrhage by vitamin K. In no group of young rats given halibut-liver oil was the incidence of fractures reduced by dosing with vitamin K. It appears, therefore, that the production of fractures in hypervitaminosis A bears no close relationship to the delayed clotting times. The results of the dicumarol experiments give further evidence that hypoprothrombinaemia can exist as a separate entity without the bones being visibly involved. The hypoprothrombinaemia of dicumarol poisoning can also be cured by vitamin K (Overman, Field, Baumann & Link, 1942).

It might be thought that the increase in the plasma/cell ratios found in the hypervitaminotic rats was only due to blood dilution subsequent to bleeding and not to a specific action of excess vitamin A. In some young rats, however, an increase in the plasma/cell ratio was not accompanied by haemorrhage. The observations that hypervitaminosis A in rats is accompanied by an increased plasma/cell ratio is in agreement with the work of Poumeau-Delille (1943) who found a severe erythroblastic anaemia in rats given toxic doses of vitamin A. In these animals there was a marked fall in both red and white blood cells, although the platelet count was normal. Poumeau-Delille states that there was no alteration in bleeding or coagulation times. This statement does not agree with the conclusions of the present work, but it must be noted that the animals in the French laboratory were given halibut-liver oil by subcutaneous injection, whereas oral administration was employed in the present investigation.

There is little in the literature suggesting any relationship between vitamins K and A in the normal animal. Clayton & Baumann (1944), studying the effect of various substances on the rate of depletion of hepatic vitamin A in rats, found that vitamin K had a slight retarding influence. As the anti-coagulant dicumarol was shown to have a similar effect on vitamin A reserves this action can hardly be regarded as specific for vitamin K. Blood and liver vitamin A levels in young chicks suffering from vitamin K deficiency were measured by Tomaszewski & Engel (1939) but their results were inconclusive.

SUMMARY

1. In addition to the well known lesions of hypervitaminosis A in rats, namely skeletal fracture and haemorrhage, the presence of a marked hypoprothrombinaemia has been confirmed.

2. The administration of vitamin K prevented this hypoprothrombinaemia and diminished the incidence of haemorrhage, although the incidence of fractures remained unchanged.

3. Massive doses of ascorbic acid did not prevent the fractures or haemorrhage in hypervitaminosis A.

4. Hypoprothrombinaemia induced in rats by dicumarol poisoning, in the absence of any excess vitamin A, was not associated with visible skeletal changes.

5. Massive doses of vitamin A caused an increase in plasma/cell ratios, which could sometimes be controlled by vitamin K.

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REFERENCES

Campbell, H. A., Smith, W. K., Roberts, W. L. & Link, K. P. (1941). *J. biol. Chem.* **138**, 1.
Clayton, C. C. & Baumann, C. A. (1944). *J. Nutrit.* **27**, 155.
Davies, A. W. (1933). *Biochem. J.* **27**, 1770.
Davies, A. W. & Moore, T. (1934). *Biochem. J.* **28**, 288.
Herbst, E. J., Pavcek, P. L. & Elvehjem, C. A. (1944). *Science*, **100**, 338.
Light, R. F., Alscher, R. P. & Frey, C. N. (1944). *Science*, **100**, 225.
Moore, T. & Wang, Y. L. (1943). *Biochem. J.* **37**, viii.
Moore, T. & Wang, Y. L. (1945). *Biochem. J.* **39**, 222.
Overman, R. S., Field, J. B., Baumann, C. A. & Link, K. P. (1942). *J. Nutrit.* **23**, 589.
Overman, R. S., Stahmann, M. A., Sullivan, W. R., Huebner, C. F., Campbell, H. A. & Link, K. P. (1942). *J. biol. Chem.* **142**, 941.
Pavcek, P. L., Herbst, E. J. & Elvehjem, C. A. (1945). *J. Nutrit.* **30**, 1.
Poumeau-Delille, G. (1943). *C.R. Soc. Biol., Paris*, **137**, 604.
Tomaszewski, von W. & Engel, C. (1939). *Z. Vitaminforsch.* **9**, 238.
Vedder, E. B. & Rosenberg, C. (1938). *J. Nutrit.* **16**, 57.
Yudkin, S. (1941). *Biochem. J.* **35**, 551.

Flocculation Tests with Electrophoretically Separated Serum Proteins

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There are now a number of flocculation tests carried out on blood serum which have proved useful indicators of hepatic dysfunction, and the mechanism involved in these tests is becoming much clearer as a result of American work with electrophoretically separated proteins. Thus the γ-globulin is very active in precipitating the gold and cephalin-cholesterol reagents (Gray, 1942; Kabat, Moore & Landow, 1942) and this fraction is also known to be increased in the serum from patients with various types of hepatitis (Gray & Barron, 1943). In both these tests albumin has an inhibitory effect and, moreover, albumin from hepatitis serum has less inhibitory power than albumin from normal serum (Kabat, Hanger, Moore & Landow, 1943; Moore, Pierson, Hanger & Moore, 1945). Very little information is available as to the effect of the α- and β-globulin fractions.

The thymol turbidity test (Maclagan, 1944*a*) appeared to be closely related to the above two tests both on clinical and chemical grounds, and the precipitate formed in the reaction was shown to be a globulin-thymol-phospholipid complex. It therefore seemed probable that a similar mechanism was operating in this test. The experiments reported here were undertaken to elucidate this point, and to investigate the α and β fractions more fully. In the meantime, Recant, Chargaff & Hanger (1945) and McCord (1945) have failed to produce a turbidity when the thymol reagent was added to pure γ-globulin solutions. A preliminary report of our experiments has already appeared (Maclagan & Bunn, 1946).

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MATERIAL

The results reported here were obtained with two collections of human serum: *N*, 40 ml. from a normal subject; *H*, 35 ml. pooled from two patients with typical infective hepatitis. Serum *N* was negative and *H* was strongly positive to all the tests used.

Preliminary experiments were also conducted on two other pooled sera with very similar results, but these will not be reported here as in these earlier separations the standard electrophoretic technique was combined with either precipitation or concentration by evaporation from the frozen state, such procedures being suspect in that they may cause modifications of the serum proteins or their complexes.

METHODS

Electrophoresis

The yields, particularly of α- and β-globulins, obtainable by the discontinuous electrophoretic technique of Tiselius (1938) (see, for example, Blix, Tiselius & Svensson, 1941) are so limited as to make a semi-continuous technique greatly preferable. Svensson (1942, 1946) has described a preparative apparatus which allows sampling and refilling to be carried out at will during the separation. For our purposes, Svensson's elaborate arrangement did not seem necessary, and a very simple modification of the standard Tiselius assembly employing the same principles was found adequate (Fig. 1).