

THE REGULATION OF THE BLOOD VOLUME IN EXPERIMENTAL NEPHRITIS. (II.)¹

By R. A. CHISOLM, *Beit Memorial Research Fellow.*

From the Pathological Department, Guy's Hospital, London, S.E.

BOTH uranium nitrate in doses of 0.010 grm. per kilo of body weight, and potassium chromate in doses of 0.035 grm. per kilo, produce in the rabbit widespread necrosis of the cells of the convoluted tubules of the kidney. In the case of either drug, anuria sets in within three or four days, and the animal dies shortly after. It has been shown by Boycott (1913¹) in the case of uranium animals, and by Chisolm (1914²) in the case of chromate rabbits, that after anuria has become established there is an alteration in the permeability of the capillary wall of such a nature as to hinder the interchange of fluid between the blood vessels and the tissues. The mechanism of the production of this alteration forms the subject of the present investigation.

Apart from the influence of retained excreta, two possible hypotheses suggest themselves: in the first place, the necrosed kidney cells may give rise to a product which is passed into the circulation, and which produces the alteration in the capillary epithelium; in the second place, the alteration in the capillary wall may be due to the direct action on the wall of the uranium nitrate or potassium chromate used to produce the nephritis. Both these hypotheses have been tested experimentally.

The method of experiment was as follows:—Nephritis was produced in rabbits of 2 kilos weight or over by the intramuscular injection of a 1 per cent. solution of uranium nitrate or potassium chromate in distilled water, the dose being 0.010 grm. per kilo of body weight in the former case, and 0.035 grm. per kilo in the latter. After anuria had set in, which was usually on the third or fourth day in the case of either drug, the animal was anaesthetised with ether, and a sample of blood taken from the carotid for the estimation of chlorides in the serum. The kidneys were then removed with aseptic precautions, and finely divided with scissors; ² they were transferred to 200 c.c. of sterile Ringer's solution (containing 0.9 per cent. NaCl, 0.042 per cent. KCl, 0.024 per cent. CaCl₂, and 0.030 per cent. NaHCO₃) in a flask. The flask

¹ [Received May 22, 1914.] The first article appeared in this Journal, vol. xviii. p. 552. Towards the expenses of this research a grant was received from the Science Committee of the British Medical Association.

² In the preliminary experiments the kidneys were ground up with sand in a sterile mortar, and extracted in the above fashion. The resulting extract filtered very slowly, and on injection into a rabbit produced immediate dyspnoea, cyanosis, and death. This method of extraction was therefore abandoned.

was then well shaken, and allowed to stand in the cold room for at least forty-eight hours. The controls were normal kidneys treated in exactly the same way. At the end of this period the extract was filtered through an ordinary filter paper, the filtrate being an opalescent fluid, more or less tinged with hæmoglobin. A normal rabbit of about 2 kilos weight was then taken, and anaesthetised with ether and urethane; the abdomen was opened, and the ureters tied at the base of the bladder. The abdomen was then closed with a few sutures. A cannula was tied in the left jugular vein, and the right carotid exposed and ligatured, and divided after a clip had been placed on the proximal end. The percentage of hæmoglobin was then estimated in a sample of blood from the carotid, and the filtered kidney extract injected in the circulation through the jugular cannula. The injected fluid was always suitably warmed; and the amount given was equal to the estimated blood volume of the animal, taken as 48 c.c. per kilo of body weight. The injection was carefully regulated so as to occupy five minutes, 4·8 c.c. of fluid per kilo being run in uniformly during each period of thirty seconds. Immediately the injection was finished the hæmoglobin was again estimated in a sample of blood from the carotid, and thereafter at five-minute intervals till fifty minutes had elapsed from the start of the injection. From the figures so obtained it is easy to calculate the relative blood volume and the amount of the injection that passes out of the vessels in each five-minute period. Throughout this paper in the tables the relative blood volume at the start is taken as 100.¹

Every time a sample of blood is taken from the carotid, a certain small amount of hæmoglobin is lost to the circulation, and this vitiates to a slight extent the calculation of the relative blood volume from the percentage of hæmoglobin. While it is possible by collecting all the samples of blood to make an approximate correction for this factor, the calculation is somewhat uncertain, and, as the error is in any case small, I have preferred to omit the correction and give the crude figures as actually observed.

NORMAL KIDNEY EXTRACT.

The results of infusing six normal rabbits with their calculated blood volume of extract of normal rabbit's kidney in Ringer's solution are shown in Table I.

TABLE I.—*Normal Kidney Extract.*

No.	Sex.	Weight.	Hæmoglobin Percentage Before Injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
LXX.	M.	2320	69	100	130	110	100	100	97	—	—	—	—	—
LXXIII.	M.	2520	74	100	145	123	119	116	112	109	106	103	100	99
LXXXIII.	M.	2060	70	100	125	113	108	108	100	97	96	—	—	—
LXXXV.	M.	1870	80	100	131	118	108	104	103	100	98	—	—	—
LXXXVII.	M.	1620	76	100	127	112	107	104	100	100	100	—	—	—
XCH.	F.	2550	82	100	132	117	114	109	105	101	100	99	—	—
Average relative volume . . .				100	131·7	115·5	109·3	106·0	102·8	100·7	100·0	—	—	—
Average per cent. of injection lost				—	68·3	16·2	6·2	3·3	3·2	2·1	0·7	—	—	—

¹ For a fuller description of the method, see Boycott, *Journ. Path. and Bacteriol.*, Cambridge, 1913, vol. xviii. p. 11.

For purposes of comparison, in Table II. are shown these results compared with the average figures, taken from my previous paper obtained by infusing four normal rabbits with normal Ringer's fluid under similar conditions.

TABLE II.

	Average Relative Blood Volume after									
	Minutes.									
	0	5	10	15	20	25	30	35	40	45
Normal rabbits } Normal Ringer }	100	143·3	125·3	113·3	107·8	105·0	102·5	101·3	100·5	100
Normal rabbits } Normal kidney extract }	100	131·7	115·3	109·3	106·0	102·8	100·7	100·0	—	—

It will be seen from Table I. that the results are fairly concordant one with another for the individual rabbits, though rabbit LXXIII. disposed of less of the injection during the first five minutes, and was somewhat slower than the others in passing the rest of the fluid out of its vessels. On the average the rabbit infused with Ringer extract of normal kidney under the conditions of experiment loses 68 per cent. of the injected fluid during the period of injection, 16 per cent. during the five-minute period following the injection, and thereafter decreasing amounts during each five-minute period, till by the end of thirty-five minutes from the start of the injection all the fluid infused has left the vessels and passed into the tissues.

Table II. shows that the kidney extract passes out of the vessels somewhat more quickly than normal Ringer's solution, but I am not disposed to lay much stress on this difference, as a similar difference was observed between my results for normal rabbits infused with normal Ringer, and those given by Boycott in his paper.

NEPHRITIC KIDNEY EXTRACT.

A. Uranium.

Six rabbits were infused with Ringer extract of nephritic kidney, the nephritis having been produced by uranium nitrate. The results are shown in Table III. and Fig. 1. Table IV. shows, for purposes of comparison, the average results so obtained; and the figures given by Boycott showing the effect on the blood volume of rabbits suffering from uranium nephritis, of infusing them with the blood volume of normal Ringer's solution.

TABLE III.—*Nephritic Kidney Extract (Uranium).*

No.	Sex.	Weight.	Hæmoglobin Percentage before Injection.	Relative Blood Volume after											
				Minutes.											
				0	5	10	15	20	25	30	35	40	45	50	
CIII. . .	F.	1970	74	100	154	139	132	128	123	119	117	116	116	116	
CIV. . .	F.	2000	72	100	150	138	133	128	126	122	120	120	120	118	
CXI. . .	F.	2250	79	100	146	136	129	125	123	120	118	116	114	111	
CXII. . .	F.	2550	65	100	155	135	130	125	123	120	118	118	116	116	
CXIII. . .	M.	2100	70	100	146	140	132	127	125	123	118	117	115	115	
CXIV. . .	M.	1970	79	100	138	120	118	116	114	113	113	111	100	109	
Average relative volume . . .				100	148.2	134.7	129.0	124.8	122.3	119.5	117.3	116.3	115.0	114.2	
Average per cent. of injection lost				—	51.8	13.5	5.7	4.2	2.5	2.8	2.2	1.0	1.3	0.8	
Average relative volume (normal)				—	131.7	115.5	109.3	106.0	102.8	100.7	100.0	—	—	—	

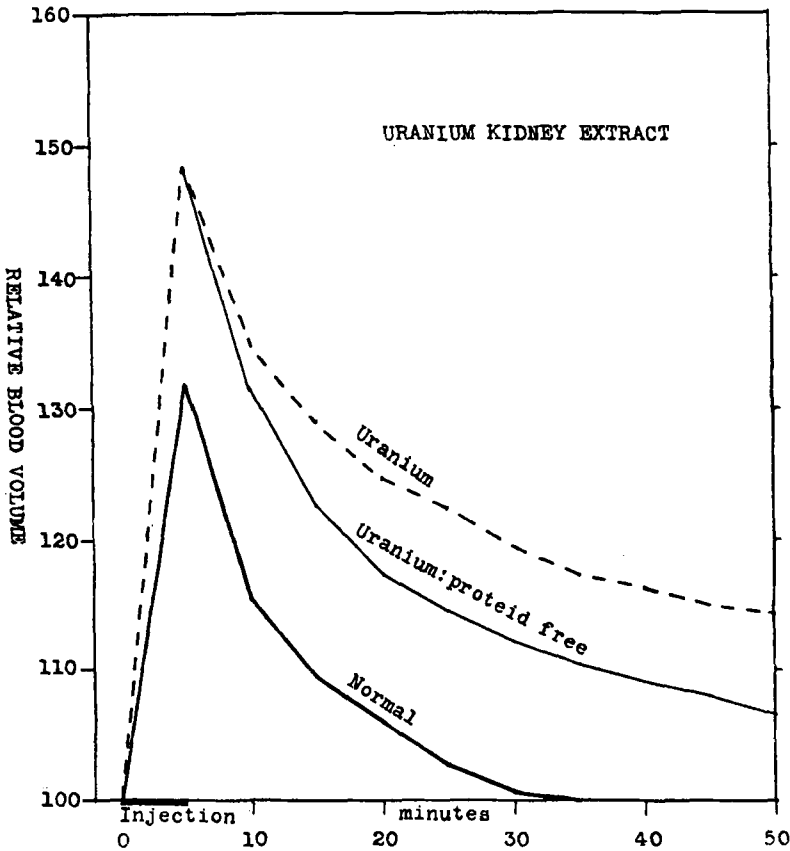


FIG. 1.

Dealing first with Table III., the results for the individual animals are fairly in agreement one with another. On an average 52 per cent. of the fluid leaves the vessels while the injection is proceeding, as compared with 68 per cent. where the fluid injected is extract of normal kidney. During the five minutes immediately following the injection, 13.5 per cent. of the fluid passes out, where nephritic kidney extract is used, while 16 per cent. passes out where normal kidney extract forms the perfusion fluid. The amount of the injection leaving the vessels becomes less in each succeeding five-minute period, and at the end of thirty-five minutes from the start, by which time all the extract of normal kidney has left the vessels, about 17 per cent. of the nephritic extract still remains in the circulation, and at the end of fifty minutes, when the experiment was stopped, 14 per cent. of the injection was still left in the vessels. Extract of uranium-nephritic kidney, therefore, under the conditions of the experiment, passes out of the circulation both more slowly and in the end less effectually than extract of normal kidney.

Table IV. shows this is also true when we compare nephritic kidney extract infused into normal rabbits with normal Ringer's solution infused into uranium-nephritic rabbits; but it is to be borne in mind that the rabbit suffering from uranium nephritis does not dispose of normal Ringer's fluid so effectually as the normal rabbit (*vide* Boycott's paper, from which the figures in Table IV. are taken).¹

TABLE IV.

	Average Relative Blood Volume after									
	Minutes.									
	0	5	10	15	20	25	30	35	40	45
Nephritic kidney extract(uranium) } Normal rabbits }	100	148.2	134.7	129.0	124.8	122.3	119.5	117.3	116.3	115.0
Normal Ringer's solution } Nephritic rabbits (uranium). — } Boycott }	100	130.4	119.3	112.4	108.0	106.8	104.9	—	—	—
Normal Ringer's solution } Normal rabbits. — } Boycott }	100	133.6	114.4	104.9	102.0	100.3	—	—	—	—

¹ The figures in Table IV. for normal rabbits perfused with normal Ringer are taken from Boycott's paper, as the normal Ringer nephritic rabbit figures came from the same source. They differ from the figures under the same heading in Table VI., which are taken from my own paper. I have used these latter figures in that instance, because the figures for the chromate rabbits infused with normal Ringer in Table VI. are from my own work. The reason for the discrepancy between Boycott's figures for normal rabbits and my own I do not know.

B. Potassium Chromate.

Six rabbits were infused with extract of potassium-chromate-nephritic kidneys in Ringer's solution. The results are shown in Table V.; Fig. 2 and Table VI. show the average results so obtained compared with the average figures, taken from my previous paper, obtained by infusing seven rabbits suffering from potassium chromate nephritis, with normal Ringer's solution.

TABLE V.—*Nephritic Kidney Extract (Potassium Chromate).*

No.	Sex.	Weight.	Hæmoglobin Percentage before Injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
LXV. . .	F.	2420	93	100	160	143	133	126	124	122	119	118	116	112
LXVI. . .	M.	1600	84	100	150	135	125	122	120	117	115	115	115	113
XCIII. . .	M.	1870	85	100	144	129	120	118	115	113	112	112	112	112
XCVI. . .	M.	1980	81	100	142	123	116	114	112	112	111	109	108	107
XCIX. . .	F.	1870	77	100	145	126	120	118	117	115	113	113	112	111
CL. . .	M.	2150	80	100	138	127	118	111	108	107	105	105	105	105
Average relative volume . . .				100	146.5	130.5	122.0	118.2	116.0	114.3	112.5	112.0	111.2	110.0
Average per cent. of injection lost				—	53.5	16.0	8.5	3.8	2.2	1.7	1.8	0.5	0.8	1.2
Average relative volume (normal)				—	131.7	115.5	109.3	106.0	102.8	100.7	100.0	—	—	—

It appears from Table V. that nephritic kidney extract (chromate) does not pass out of the circulation so completely or so quickly as normal kidney extract. During the injection period 53.5 per cent. of nephritic extract passes out as compared with 68 per cent. of normal kidney extract, and by the end of thirty-five minutes from the start, by which time all the normal extract has passed into the tissues, 12.5 per cent. of the nephritic extract still remains in the vessels, and when the experiments came to an end at fifty minutes from the start, 10 per cent. of the injection still remained in the circulation.

Table VI. shows that when extract of chromate-nephritic kidney is infused into a normal rabbit, the subsequent course of events is much the same as when normal Ringer's solution is infused into rabbits suffering from potassium-chromate nephritis, the injection in both cases passing out of the vessels both less quickly, and in the end less effectually, than when a normal rabbit is infused with Ringer's solution or with extract of normal kidney.

In the case, therefore, of both uranium-nitrate and potassium-chromate nephritis, Ringer's extract of nephritic kidney when infused into a normal rabbit passes out of the circulation at a rate and to an extent which is comparable with the rate and extent to which normal Ringer's solution leaves the vessels of a nephritic rabbit, whether the

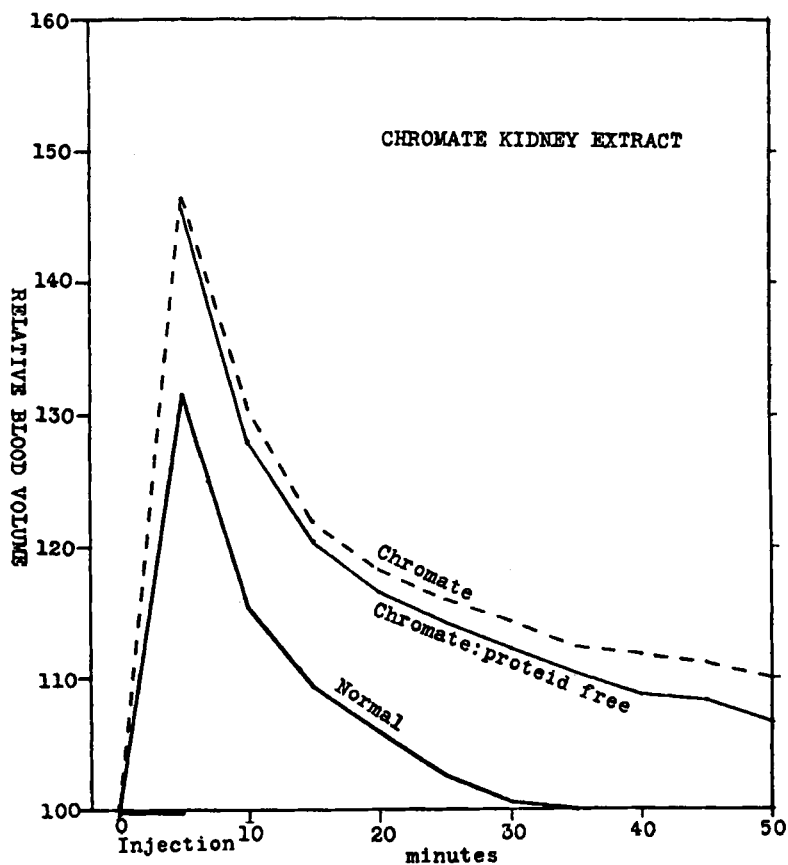


FIG. 2.

TABLE VI.

	Average Relative Blood Volume after										
	Minutes.										
	0	5	10	15	20	25	30	35	40	45	50
Nephritic Kidney extract (chromate) rabbits	100	146.5	130.5	122.0	118.2	116.0	114.3	112.5	112.0	111.2	110.0
Normal Ringer's solution Nephritic rabbits (chromate)	100	156.7	133.4	124.3	118.4	115.4	113.4	112.4	112.4	112.4	112.4
Normal Ringer's solution Normal rabbits. —Chisolm	100	143.3	125.3	113.3	107.8	102.5	101.3	100.5	100	100	—

Nor is it due to the presence in the nephritic kidneys of uranium nitrate or potassium chromate, which passes into solution in Ringer's fluid with which the extract is made, and alters the permeability of the capillaries by direct action on their wall. Two facts are against this supposition. In the first place, Mr. Rogerson, who kindly tested the matter for me, tells me that the potassium-chromate kidneys contain only a trace of the salt, and none at all could be found in the extract. The uranium kidneys were not tested for the presence of uranium nitrate. In the second place, we have the results set out in Tables IX. and X. Table IX. shows the alteration in blood volume produced by the infusion of Ringer's solution in the usual way into rabbits which about fifteen minutes previously had received an intravenous injection of 0.010 grm. per kilo of uranium nitrate, the dose used for nephritis. For comparison the bottom line shows the figure for normal untreated rabbits infused with Ringer's solution. Table X. shows the results in rabbits treated in the same way after the intravenous injection of 0.035 grm. potassium chromate per kilo. The only alteration that such treatment produces in either case is that in the case of the chromate animals in Table X. during the injection period rather less of the injection fluid passes out of the vessels than in untreated animals infused with normal Ringer, a difference which may be due to an alteration in blood pressure. The difference, however, is soon made up, and in both cases the fluid leaves the vessels as quickly and completely as in the case of the normal animal.

It would seem, therefore, that Ringer's solution extracts from the nephritic kidney some substance which prevents the extract passing out of the circulation so quickly as extract of normal kidney or normal Ringer's solution.

TABLE IX.—*Intravenous Injection of Uranium Nitrate: 0.010 Grm. per Kilo, followed by Injection of Ringer's Solution.*

No.	Sex.	Weight.	Hæmoglobin Percentage before Injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
CXXXVI.	F.	2350	82	100	137	119	111	104	99	98	-	-	-	-
CXL.	M.	1950	79	100	141	120	110	105	101	100	99	-	-	-
CXLIII.	F.	2270	75	100	139	121	113	107	104	101	99	97	-	-
Average relative volume				109	139.0	120.0	111.3	105.3	101.3	99.7	(99)	(97)	-	-
Average per cent. of injection lost				-	61.0	19.0	8.7	6.0	4.0	1.6	0.7	-	-	-
Average relative volume (normal Ringer)				-	143.3	125.3	113.3	107.8	105.0	102.5	101.3	100.5	100.0	-

Some experiments were performed to decide whether this substance was of the nature of a coagulable proteid, or so closely connected with

it as to be inseparable from it by ordinary methods, or whether it was incoagulable by heat. To this end extracts of both uranium and chromate kidneys were made faintly acid with 2 per cent. acetic acid boiled, filtered repeatedly till clear, and made up to the volume of the original extract with Ringer's fluid, after being made faintly alkaline with 2 per cent. sodium carbonate solution. The fluid so obtained was then infused into rabbits of about 2 kilos weight in the usual way.

TABLE X.—*Intravenous Injection of Potassium Chromate: 0.035 Grm. per Kilo, followed by the Injection of Ringer's Solution.*

No.	Sex.	Weight.	Hemoglobin Percentage before Injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
CLVI.	F.	2250	68	100	148	124	106	101	99	97	-	-	-	-
CLVII.	M.	1980	73	100	149	128	110	106	103	100	99	-	-	-
CLVIII.	F.	2370	67	100	159	129	114	105	100	99	-	-	-	-
Average relative volume				100	152.0	127.0	110.0	104.0	100.7	98.7	-	-	-	-
Average per cent. of injection lost				-	48.0	25.0	17.0	6.0	3.3	2.0	-	-	-	-
Average relative volume (normal Ringer)				-	143.3	125.3	113.3	107.8	105.0	102.5	101.3	100.5	100	-

TABLE XI.—*Nephritic Kidney Extract (Uranium—Proteid-free).*

No.	Sex.	Weight.	Hemoglobin Percentage before Injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
CXVIII.	F.	1900	78	100	144	134	124	115	111	108	107	107	107	105
CXXIV.	F.	2270	75	100	139	123	115	109	106	104	103	101	100	100
CXXV.	M.	2120	73	100	159	143	133	128	124	122	118	116	114	114
CXXIX.	M.	2020	71	100	165	142	134	129	127	122	118	116	113	111
CXXXII.	F.	2080	84	100	143	124	114	109	108	108	108	106	106	105
CXXXIII.	M.	2180	83	100	143	125	117	114	112	109	108	108	108	106
Average relative volume				100	148.8	131.8	122.8	117.3	114.7	112.2	110.3	109.0	108.0	106.8
Average per cent. of injection lost				-	51.2	17.0	9.0	5.5	3.6	2.5	1.9	1.3	1.0	1.2
Average relative volume (normal)				-	131.7	115.5	109.3	106.0	102.3	100.7	100.0	-	-	-

The results are seen in Figs. 1 and 2, and Tables XI. and XII. It will be seen that in the case of both uranium and chromate proteid-free extracts, the amount of fluid that passes out of the circulation during the injection period is the same as where untreated nephritic kidney extract is used for infusion, but that ultimately rather more of

the proteid-free extract passes out than of the extract in which the coagulable proteid still remains. In neither case, however, does the blood at the end of fifty minutes from the start return to its original volume. It would seem, therefore, that in getting rid of the coagulable proteid (and the filtrate was so far free of it that it gave no precipitate with salicyl-sulphonic acid in saturated solution), one gets rid to a slight extent of the substance which retards the passage of fluid from the vessels, but by no means all of it, a considerable amount remaining behind in the proteid-free fluid. Possibly the amount that is lost is carried down mechanically by the precipitated proteid.

TABLE XII.—*Nephritic Kidney Extract (Potassium Chromate—Proteid-free).*

No.	Sex.	Weight.	Hemoglobin percentage before injection.	Relative Blood Volume after										
				Minutes.										
				0	5	10	15	20	25	30	35	40	45	50
CXXXV.	M.	2250	87	100	143	130	113	110	109	107	106	106	105	105
CXLVII.	M.	2450	76	100	146	129	123	119	115	113	110	109	107	106
CXLVIII.	F.	2550	82	100	141	120	120	114	112	111	109	108	108	106
CL.	M.	1950	72	100	151	132	127	122	119	117	115	113	113	111
CLII.	M.	1820	70	100	149	125	121	119	117	115	113	109	108	106
CLIII.	M.	1700	93	100	141	126	119	116	113	111	109	108	108	107
Average relative volume				100	145.2	128.0	121.3	116.7	114.2	112.3	110.3	108.8	108.2	106.7
Average percent. of injection lost				—	54.8	17.2	6.7	4.6	2.5	1.9	2.0	1.5	0.6	1.5
Average relative volume (normal)				—	131.7	115.5	109.3	106.0	102.8	100.7	100.0	—	—	—

CONCLUSIONS.

Extracts made with Ringer's fluid from uranium-nitrate or potassium-chromate-nephritic kidneys, but not from normal kidneys, contain a substance which hinders the passage of fluid from the vessels to the tissues when infused into rabbits. This substance is not of the nature of a coagulable proteid.

REFERENCES.

1. BOYCOTT. *Journ. Path. and Bacteriol.*, Cambridge, 1913, vol. xviii. p. 11.
2. CHISOLM *Ibid.*, pp. 404, 552.
3. AMBARD AND WEIL. . . . *Journ. Path. et Physiol. gen.*, Paris, 1912, tome xiv. p. 253.