THE PASSAGE OF PROTEINS FROM THE VASCULAR SYSTEM INTO JOINTS AND CERTAIN OTHER BODY CAVITIES* †

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Investigations designed to elucidate the manner of exchange of substances in solution between the joints and the vascular system have been concerned, for the most part, with the passage of materials from the joint space to the blood stream, the lymphatics, or the subsynovial tissue spaces. Of equal importance for a more complete understanding of normal joint physiology is knowledge concerning the passage of various substances from the vascular system into the joint space.

Previous studies have demonstrated that proteins contained in egg white and horse serum are removed from the knee joints of dogs solely by way of the lymphatics (1), while in the cat certain drugs of small molecular dimensions in aqueous solutions are removed from joints chiefly by way of the blood capillaries (2). The present experiments were undertaken to study the transference of proteins from the vascular system into joints. In addition, in each experiment the passage of protein into the aqueous humor, the spinal fluid, and the urine was investigated.

Materials and Methods

Normal albino rabbits varying between 3½ and 5 pounds in weight were used in all experiments. Following the withdrawal of a control blood sample, the material to be employed, namely crystalline egg albumin, the albumin or euglobulin fractions of horse serum, or whole normal horse serum was injected into a marginal ear vein. After varying intervals of time each rabbit was rapidly exsanguinated. Immediately thereafter, specimens of synovial fluid, aqueous humor, urine, and spinal fluid were obtained, in the order mentioned; usually not more than 10 minutes was required for these procedures.

In order to avoid the danger of blood contamination, the skin overlying the knee joints was removed and the periarticular tissues cauterized. A 25 gauge hypodermic needle attached to a tight fitting syringe containing 0.5 cc. of physiological saline solution

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was inserted into the joint space through the cauterized area, at such an angle that the point of the needle came to rest beneath the patella. This procedure was facilitated by lifting the patella forward by traction upon the infrapatellar ligament. The saline was then injected into the joint, and without removing the needle the contents of the joint space were aspirated into the syringe.

Aqueous humor was removed from each eye. The conjunctival sacs were repeatedly washed with saline and finally with 95 per cent ethyl alcohol. A 25 gauge hypodermic needle attached to a tuberculin syringe was passed obliquely through the non-vascular cornea and the contents of the anterior chamber (0.15 to 0.2 cc.) withdrawn. The eye was fixed during this operation by grasping the conjunctival membrane with a fine-tooth forceps.

Samples of urine were aspirated from the urinary bladder through a cauterized portion of the wall. The fluid was neutralized with the requisite acid or alkali and any turbidity or precipitate removed by centrifugation.

Spinal fluid (0.2 to 0.5 cc.) was withdrawn from the cisterna magna through a 25 gauge hypodermic needle after the skin and external tissues had been removed and the remaining tissues cauterized.

In spite of the care taken in collecting the specimens, contamination with blood occurred occasionally. Errors from this source were avoided in the following manner. Smears of each fluid obtained were stained with Wright's stain. The amount of blood contamination was estimated by noting the proportion of erythrocytes to the normal cellular constituents. The presence of blood was further determined by centrifugation of each body fluid specimen and examination of the sediment, if any. The minimum of blood required to give rise to positive precipitative reactions was determined by intentional contamination of measured quantities of fluid. Specimens containing blood in amounts sufficient to invalidate the result of precipitative tests were discarded.

Samples of the above mentioned body fluids and the blood serum were set up in appropriate dilutions against the specific antiserum for the material employed, the tests for precipitinogen being done by the interfacial ring method. Readings were made after 2 hours of incubation at room temperature. In each experiment a control blood serum sample taken before the injection of foreign protein was set up against the antiserum employed, as well as saline controls. Each antiserum was tested frequently against dilutions of the homologous antigen in order to be certain that its potency had not diminished.

Preparation of Protein Solutions.—Crystalline egg albumin[1] (stored in saturated $(NH_4)_2 SO_4$ solution at 4°C.) was dialyzed against distilled water until it was sulfate-free and then kept at 4°C. The strength of the solution of egg albumin employed for immunization purposes was 0.98 per cent. Solutions of greater concentration (16.7 or 7.6 per cent) attained by the method of Thalhimer (3) and made isotonic by the addition of sodium chloride were used in the experiments.

Horse serum albumin and euglobulin fractions[2] were prepared according to the

[1] We are indebted to Dr. John F. Enders for the crystalline egg albumin. This protein was prepared by Miss M. McLanahan under the supervision of Dr. Edwin J. Cohn, in the Department of Physical Chemistry, The Harvard Medical School.

[2] For the preparation of these protein fractions we are indebted to Dr. Marian Ropes of the Massachusetts General Hospital.

method of Doerr and Berger (4) in the manner previously described (1). The total protein concentration of the aqueous serum albumin preparation thus obtained and employed for immunization purposes was 1.7 per cent. Prior to intravenous injection, this solution was made isotonic by the addition of sodium chloride. The amount injected in any given experiment was calculated to be the equivalent of the albumin contained in 2 cc. of normal horse serum. The strength of euglobulin solution (dialyzed against 4 per cent NaCl solution) employed for immunization and for the experiments was 1.6 per cent.

Preparation of Immune Sera.—Immune rabbit sera of high precipitating potency were prepared by the method of Dienes and Schoenheit (5). Successive doses (0.2 cc.) of antigen were injected into 1 to 2 week old subcutaneous tuberculous lesions. These were produced by injecting 0.5 cc. of a turbid suspension of a 1 month old culture of tubercle bacilli of low virulence (strain H 37, human type).

The highest dilutions of antigen detectable by precipitation with antisera *versus* egg albumin and horse serum proteins are given in footnotes in the individual tables for each group of experiments employing a given type of foreign protein.

RESULTS

(*a*) *Experiments Employing Crystalline Egg Albumin*

Fourteen rabbits were injected intravenously with egg albumin in varying dosage. At different time intervals the body fluids were examined for the presence of the foreign protein. The results are given in Table I. The actual volumes of solution (never exceeding 3 cc.) administered are not recorded since several preparations of differing protein concentration were utilized.

The marked variation in the initial titer of the blood serum (specimens obtained within 10 minutes after injection) was due chiefly to the difference in dosage of the inoculum. Thus rabbit 7-36 received 0.25 cc. of 7.6 per cent egg albumin solution, whereas rabbits 7-35 and 7-33 received 2.0 cc. of the same preparation. Animals receiving small doses of protein (0.2 to 0.3 cc.) had an initial blood serum titer varying between 1/750 and 1/1500. With larger doses (1.0 to 2.0 cc.) the initial titer of the serum varied between 1/2000 and 1/6000.

The egg albumin[3] was found to be transferred from the vascular system into the knee joint fluids and aqueous humors, within 5 minutes after its injection. The foreign protein concentration in these body fluids tended to vary directly with the concentration in the serum. Differences were

[3] Because of the general agreement that crystalline egg albumin is a homogeneous protein of well defined properties, and the recent demonstration (6), that the associated carbohydrate is almost certainly an actual part of the protein molecule, there is little doubt that in the present experiments the substance detected was egg albumin and not a contaminating material of a smaller molecular size.

encountered, however, among individual animals and occasionally between the two joint washings or aqueous humors of the same animal. Traces of egg albumin were detected in the undiluted spinal fluid in 2 of 12 experi-

TABLE I

Results of Experiments in Which Crystalline Egg Albumin Was Injected Intravenously

Rabbit No.	Duration of experiment	Titer of injected protein in blood serum				Titer of injected protein in body fluids					
		Initial*	Intermediate		Terminal	Joint fluids (saline washings)†		Aqueous humor		Spinal fluid	Urine
						Right	Left	Right	Left		
	min.										
7-36	5				750	1	3	1	0	0	100
7-35	5				6000	6	20	160	80	0	1500
7-33	$6\frac{1}{2}$				5000	5	10	20	80	—	1500
7-52	10				3000	3	3	1	1	—	750
7-37	11				1000	0	1	0	0	0	50
7-51	28				2000	3	12	6	6	1	500
7-81	30	1000			750	1	1	1	1	0	2000
	hrs.										
7-80	1	2000			250	1	1	1	1	0	2000
7-00	1	3000			1000	6	6	6	6	0	5000
7-94	2	3000			500	6	1	6	6	1	5000
2-56	4	2000			80	1	6	1	1	0	960
2-53	6	2000			20	1	1	1	1	0	160
7-82	24	1500	1 hr.	250	1	0	0	0	0	0	0
			6 hrs.	20							
4-03	25	1000	1 hr.	250	2	0	0	0	0	0	6
			6 hrs.	10							

The anti-egg albumin serum employed in these experiments gave positive precipitative tests with dilutions of homologous antigen up to 1/2,000,000.

* The term initial as employed in this and succeeding tables refers to the titer obtained on blood samples taken within the first 10 minutes after the intravenous injection of the foreign protein.

† The specimens of joint fluid (referred to as saline washings) are washings of the joint cavity and represent an estimated initial five or tenfold dilution which has not been corrected for in the table.

ments in which suitable samples were procured; these were from rabbits tested 28 and 120 minutes respectively after injection. There was a very rapid reduction in the concentration of egg albumin in the serum. In experiments lasting 24 and 25 hours, only the undiluted serum or at most a twofold dilution gave positive precipitative tests. In such instances it was no longer detectable in the knee joint washings or aqueous humors. The rapid entrance and relatively speedy removal of egg albumin from these

body cavities suggest that the barriers separating these spaces from the vascular system are more or less freely permeable to this protein. Much of the egg albumin was quickly eliminated *via* the kidneys as shown by the high concentration in the urine (see Table I). The almost uniformly higher titer of the urine as compared with that of the blood, 1 hour following the injection, was probably due to the relative volumes of the two. Such findings further suggest that the egg albumin molecule was excreted *via* the kidneys without marked alteration in its serological reactivity.

(*b*) *Experiments Employing Horse Serum Albumin*

Comparable experiments were carried out in 14 rabbits injected with solutions of the albumin fraction[4] of horse serum. These results are summarized in Table II. It will be noted that the appearance time of this larger antigen in the knee joints and aqueous humors was somewhat longer than that required for egg albumin.

It is apparent that the concentration of foreign protein in the knee joint fluid is nearly always greater than in the aqueous humor (see footnote Table II). In the experiments of longer duration (24 hours or more) the antigen present in the joints continued to increase in concentration, whereas it was detectable in only 4 out of the 8 specimens of aqueous humor. In these 4 instances the concentration was no greater than in those of the rabbits examined after 1 hour. The antigen of the serum albumin fraction was detected in the undiluted spinal fluid in only 1 of the 13 specimens tested. In the urine it was present in 5 of the 14 samples although never in very great concentration. Following an initial drop during the first hours, the concentration of antigen in the blood remained relatively high for as long as 170 hours, the level attained at 24 hours being thereafter depressed only very slowly. It is of considerable interest that in rabbit 8-10, examined 7 days after injection, a free antigen was present in the circulating blood simultaneously with antibody which had been developed by the animal in response to the albumin complex. In this case antigen was present in

[4] It is now known that the albumin fraction of horse serum as usually prepared is a mixture of several substances (7, 8). Through the kindness of Dr. F. E. Kendall, samples of seroglycoid and two crystalline albumin fractions of different carbohydrate content were obtained and tested against the antiserum. The crystalline albumin of greater carbohydrate content (A) similar to Kekwick's (8) more soluble fraction, reacted to practically the same titer as our whole albumin fraction, whereas the carbohydrate-poor crystalbumin (B) and seroglycoid reacted only in very low dilutions. These findings suggest that the material detected in these experiments was the single crystalline albumin (A).

the joints in greater amount than in any animal of the series previously tested. The significance of these findings will be discussed subsequently.

TABLE II

Results of Experiments in Which Horse Serum Albumin Was Injected Intravenously

Rabbit No.	Duration of experiment	Titer of injected protein in blood serum			Titer of injected protein in body fluids					
		Initial	Intermediate	Terminal	Joint fluids (saline washings)*		Aqueous humor		Spinal fluid	Urine
					Right	Left	Right	Left		
	min.									
7-30	11			1000	0	0	0	0	0	1
7-05	12			750	0	0	1	0	—	6
7-50	23			1000	1	1	1	1	0	0
7-53	26			500	0	1	0	0	0	0
7-04	27			1000	1	1	3	1	0	3
7-31	40			750	1	1	0	0	0	0
	hrs.									
7-01	1	200		200	1	1	1	1	0	0
7-95	2	750		500	1	1	1	1	0	0
2-57	4	750		750	1	3	1	1	0	0
2-54	6	500		250	3	6	1	1	1	0
7-83	24	750		100	1	3	1	1	0	0
4-04	25	100	2 hrs. 100 6 " 80	80	1	1	0	0	0	0
8-11†	76	250	25 " 250 72 " 250	250	6	6	1	0	0	25
8-10†‡	170	750	25 " 250 72 " 250 144 " 250	250	12	12	1	0	0	1

The anti-horse albumin serum employed in these experiments gave positive precipitative tests with dilutions of homologous antigen up to 1/870,000, with dilutions of horse serum euglobulin antigen up to 1/310, and with dilutions of normal horse serum up to 1/20,000.

* The specimens of joint fluid (referred to as saline washings) are washings of the joint cavity and represent an estimated initial five or tenfold dilution which has not been corrected for in the table.

† The joint washings showed no reaction with rabbit immune serum for horse serum euglobulin.

‡ The blood serum at death contained precipitins for horse serum albumin reacting in antigen dilutions up to 1/100.

(*c*) *Experiments Employing Horse Serum Euglobulin*

Horse serum euglobulin[5] was used in 9 experiments varying in duration from 17 minutes to 148 hours (see Table III). The rate of passage of this

[5] It is probable that the euglobulin used does not represent a homogeneous molecular species comparable to that of the crystalline egg albumin. It is, however, almost certain

protein from the vascular system into the knee joints and anterior chambers was less rapid than that of egg albumin. Little if any difference was ob-

TABLE III

Results of Experiments in Which Horse Serum Euglobulin Was Injected Intravenously

Rabbit No.	Duration of experiment	Titer of injected protein in blood serum			Titer of injected protein in body fluids					
		Initial	Intermediate	Terminal	Joint fluids (saline washings)*		Aqueous humor		Spinal fluid	Urine
					Right	Left	Right	Left		
	min.									
8-04	17			1500	0	1	0	0	0	0
7-38	18			1000	0	0	0	0	0	0
7-39	23			1000	1	1	0	0	0	0
	hrs.									
7-34	2	1000		750	1	3	1	1	0	0
8-03	2	1000		1000	1	6	1	1	0	0
8-01	24	1000	2 hrs. 1000	250	6	6	3	3	0	0
			6 " 500							
8-02	24	1000	2 " 1000	250	6	3	3	3	0	0
			6 " 750							
8-13†	76	1000	24 " 500	100	6	6	1	1	0	3
			48 " 250							
			72 " 250							
8-12‡	148	1000	24 " 250	0	0	0	0	0	0	0
			48 " 250							
			72 " 250							
			96 " 100							
			144 " 0							

The anti-horse euglobulin serum employed in these experiments gave positive precipitative tests with dilutions of homologous antigen up to 1/465,000, with dilutions of horse serum albumin antigen up to 1/5800, and with dilutions of normal horse serum up to 1/15,000.

* The specimens of joint fluid (referred to as saline washings) are washings of the joint cavity and represent an estimated initial five or tenfold dilution which has not been corrected for in the table.

† The blood serum and joint washings showed no reaction with rabbit immune serum for horse serum albumin.

‡ The blood serum at death contained precipitins for horse serum euglobulin reacting in antigen dilutions up to 1/62,000. This finding accounts for the absence of foreign protein from all samples of the body fluids at the termination of the experiment.

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that in studying the entrance of the euglobulin fraction with the aid of its corresponding antiserum we were demonstrating the presence of substances distinct from those in the horse albumin fraction (footnote Table III). These substances are in all likelihood proteins, since Coghill and Creighton (9) have shown that the carbohydrate associated with horse globulin is serologically inert.

served between the rates of passage of horse albumin and euglobulin antigens. Although there was a considerable diminution in the concentration of horse euglobulin in the blood stream in the first 24 hours, the fall in titer was subsequently slow until immunization had taken place (rabbit 8-12). In the experiments of 24 to 76 hours' duration the concentration of the foreign protein in the joint fluids was more uniformly high than in the experiments of 2 hours or less and was usually higher than the concentration in the aqueous humors. No horse serum euglobulin was detected in the spinal fluid of any of the 9 rabbits used. In only 1 rabbit (No. 8-13), examined 76 hours after injection, was it demonstrable in the urine, and here the highest dilution yielding a precipitate with antiserum was 1/3. In view of the positive findings obtained with the joint washings and aqueous humors of this animal (No. 8-13), it was surprising to find on testing rabbit 8-12 at 148 hours that no antigen was demonstrable in any of the body fluids. However, when the blood serum of the latter was tested for the presence of antibodies to horse euglobulin, such precipitins were found in moderately high titer. The implications of this will be considered later.

(*d*) *Experiments Employing Whole Normal Horse Serum*

Although the previous experiments had shown the ready passage of horse serum albumin and euglobulin antigens into the knee joints and anterior chambers, it seemed desirable to learn whether or not whole horse serum which had not been subjected to fractionation would exhibit similar properties. In 6 experiments of 1 to 5 hours' duration, in which 10 cc. of whole horse serum was injected intravenously, it was found that antigenic constituents passed regularly from the blood into the knee joints and anterior chambers (see Table IV). No horse serum antigen could be demonstrated in the urine of any animal in this series. In 2 of the 6 experiments, the undiluted spinal fluid gave positive precipitative reactions; the remaining 4 spinal fluids were negative.

A smaller dose (1 cc.) of normal horse serum was subsequently employed in 8 experiments of 2 and 4 hours' duration (Table IV). Again, with the exception of one eye in each of 2 rabbits (Nos. 5-0 and 5-4), horse serum constituents were demonstrated to have passed into the joint fluids and aqueous humors, but none could be shown in either the urine or spinal fluid. In another group of 6 experiments lasting from 4 to 121 hours, 2 cc. of whole horse serum were injected intravenously (Table V). In order to obtain more information regarding the relative concentration in the body fluids of those fractions which had previously been employed separately, the pre-

cipitative tests were performed with antiserum directed against whole horse serum as well as with anti-albumin and anti-euglobulin sera.

It will be noted that for the initial samples of blood serum, the highest dilutions yielding positive reactions with antisera for whole horse serum and horse serum euglobulin were greater than those positive with immune

TABLE IV

Results of Experiments in Which Whole Normal Horse Serum Was Injected Intravenously

Rabbit No.	Dosage of horse serum	Duration of experiment	Titer of injected proteins in body fluids						
			Blood serum terminal	Joint fluids (saline washings)*		Aqueous humor		Spinal fluid	Urine
				Right	Left	Right	Left		
	cc.	*hrs.*							
N	10	1	1000	3	3	1	1	0	0
M	10	2	1200	6	6	6	6	0	0
K	10	4	500	3	1	1	1	0	0
J	10	4	600	3	3	1	1	0	0
I	10	4	320	1	1	1	1	1	0
L	10	5	2000	3	3	3	3	1	0
5-0	1	2	500	1	1	1	0	0	0
5-4	1	2	500	1	3	0	1	0	—
3-4	1	2	300	1	3	1	1	0	0
5-3	1	2	300	1	1	1	1	0	0
3-2	1	2	300	1	1	1	1	0	0
4-5	1	4	300	1	1	1	1	0	0
5-5	1	4	200	1	1	3	3	0	0
3-9	1	4	100	1	1	1	1	0	0

The antiserum employed in these experiments gave a positive precipitative test with dilutions of normal horse serum up to 1/18,000.

* The specimens of joint fluid (referred to as saline washings) are washings of the joint cavity and represent an estimated initial five or tenfold dilution which has not been corrected for in the table.

serum *versus* the horse albumin fraction; this relation persisted in most of the subsequent samples in which the titers against each antiserum slowly fell. The horse serum antigens were regularly demonstrated in the joint washings, with the titers of the euglobulin and albumin constituents found there bearing a relationship similar to that obtaining in the blood serum. In the majority of specimens of aqueous humor obtained within the first 24 hours, the concentration of the horse serum antigens was less than that in the joint washings. In the two experiments of longer duration no foreign protein could be detected in the aqueous humor. No horse serum antigen was found in any spinal fluid or urine specimen.

TABLE V

Results of Experiments in Which Whole Normal Horse Serum Was Injected Intravenously (2 Cc. Dosage) Precipitin Tests Were Performed with Antisera for Whole Horse Serum, Horse Serum Euglobulin, and Horse Serum Albumin

Rabbit No.	Duration of experiment	Proteins tested for with specific antisera	Titer of injected proteins in blood serum					Titer of injected proteins in body fluids					
			Initial	6 hrs.	24 hrs.	90 hrs.	Terminal	Joint fluids (saline washings)*		Aqueous humor		Spinal fluid	Urine
								Right	Left	Right	Left		
	hrs.												
8-08	4	Whole horse serum	1000				750	12	12	1	0	0	0
		H.S. euglobulin	1000				750	6	6	0	0	0	0
		H.S. albumin	250				50	3	3	0	0	0	0
8-09	4	Whole horse serum	1000				750	6	6	1	1	0	0
		H.S. euglobulin	1000				1000	6	6	1	3	0	0
		H.S. albumin	100				50	1	1	0	0	0	0
8-05	24	Whole horse serum	1000	750			250	3	6	1	1	0	0
		H.S. euglobulin	1000	750			500	3	6	1	1	0	0
		H.S. albumin	250	100			50	1	3	1	0	0	0
8-07	24	Whole horse serum	1000	500			250	3	3	1	1	0	0
		H.S. euglobulin	1000	500			250	3	1	3	1	0	0
		H.S. albumin	100	100			50	1	0	0	0	0	0
4-14†	120	Whole horse serum	750		250	250	250	12	6	0	0	0	0
		H.S. euglobulin	1000		750	250	250	6	6	0	0	0	0
		H.S. albumin	500		100	100	100	12	6	0	0	0	0
4-16†	121	Whole horse serum	750		250	100	100	6	12	0	0	0	0
		H.S. euglobulin	1000		500	250	100	3	6	0	0	0	0
		H.S. albumin	250		100	100	100	6	6	0	0	0	0

The titers of the antisera *versus* horse serum albumin and euglobulin employed in these experiments are given in footnotes in Tables II and III. The antiserum *versus* normal horse serum employed gave positive precipitative tests with dilutions of homologous antigen up to 1/40,000, with horse serum euglobulin in dilutions up to 1/155,000, and with horse serum albumin in dilutions up to 1/580,000.

* The specimens of joint fluid (referred to as saline washings) are washings of the joint cavity and represent an estimated initial five or tenfold dilution which has not been corrected for in the table.

† The blood serum at death contained no precipitins for horse serum, horse serum euglobulin, or horse serum albumin reacting with antigen dilutions of 1/10 or higher.

DISCUSSION

From the data presented in the foregoing experiments it is apparent that foreign proteins of varying molecular size pass from the vascular system into the joint cavity in relatively short periods of time. The smaller molecule of egg albumin appeared in the synovial fluid in detectable quantities sooner than did the larger proteins contained in horse serum. These differences are obvious even when allowance is made for the fact that the anti-egg albumin serum could detect homologous antigen in higher dilution than the antisera *versus* the horse serum proteins. The results further show that the smaller egg albumin molecule is more readily removed from the joint spaces than are the larger horse serum proteins which show a tendency to increase in concentration.

If homologous serum proteins behave in a manner similar to foreign proteins, then these observations concerning the passage of proteins into joints are of significance in their application to normal joint physiology. The colloids contained in synovial fluid increase its osmotic pressure, thereby counteracting part of the osmotic pressure of the blood and favoring the diffusion of plasma water into the joint. In view of the observed differences between the rate of entrance and removal of foreign proteins it is likely that the entrance of homologous plasma proteins into the normal joint is slow, else the rapid accumulation of protein would result in an effusion. The relatively slow removal of proteins derived from serum may explain the high protein concentrations found in pathological joint effusions of long duration. Variations in permeability between two knee joints of the same animal apparently occur frequently and must represent local and presumably temporary changes. Similar variations occurring from time to time might account for the differences in total proteins found in normal synovial fluids (10).

The permeability of synovial membrane to protein is a factor which conditions the feasibility of treating joint infections with specific antisera either passively or by active immunization, since it has been demonstrated in human beings that antibodies may be found in joints (11–13). The present experiments reported here not only have a bearing on this problem but in addition suggest further studies concerning articular tissue hypersensitivity, as for example, the allergic joint manifestations of serum sickness (14).

In the great majority of our experiments (49 out of 54) no horse serum proteins or egg albumin were detected in the spinal fluid; in the 5 animals in which foreign protein was found, the concentration was 0.05 to 0.4 per

cent of that of the blood. This is in accord with the results of other workers (15–21) who have found the concentration of certain immune bodies, toxins, viruses, and dyes in the spinal fluid to represent only a small fraction of that in the serum. The fact that egg albumin was not detected in spinal fluid any oftener than were horse serum proteins indicates that the passage of protein into the spinal fluid is dependent upon factors other than molecular size.

The passage of each type of protein from the blood into the aqueous humor suggests that the separating barrier is more permeable than that of the choroid plexus. This finding is in agreement with that of previous workers, namely, that minute amounts of protein are present in normal aqueous humor (22, 23) and that traces of immune substances are detectable in the aqueous humor of highly immunized animals (24, 25). The rate of entrance of the individual proteins was found to vary slightly according to the size of the molecule, egg albumin entering the aqueous humor most readily and horse serum euglobulin most slowly. The failure of egg albumin or horse serum albumin to accumulate in the aqueous humor in the longer experiments implies that the rate of removal of these proteins must approach their rate of entrance.

The data dealing with the rate of removal of injected protein from the blood of normal rabbits confirm the findings of earlier workers that crystalline egg albumin is eliminated rapidly (26–30), while the constituents of horse serum disappear much more slowly (27, 29, 31–35). It was observed that rabbits injected with small quantities of horse serum showed a considerably higher blood stream titer, 2 to 4 hours after inoculation, than might be expected by comparison with the titers of the blood in animals of similar size receiving large doses (10 cc.). In agreement with the experiments of Dean, Goldsworthy, and TenBroeck (34) with normal serum and those of Goodner, Horsfall, and Bauer (36) using antipneumococcal serum, our results suggest that certain possibly aggregated constituents of whole horse serum may under proper conditions undergo dissociation in the body of the rabbit. The sudden disappearance of antigen from the blood together with the concomitant presence of circulating precipitins in rabbit 8-12, 6 days after injection of horse euglobulin, is further evidence for the belief that antibody and homologous free antigen rarely if ever exist uncombined in the blood. In rabbit 8-10, injected with the horse albumin fraction, antibody was found present in the blood simultaneously with a free antigen. Such results are probably due to the multiplicity of antigens in the inoculum, the animal having produced antibodies to one of the

constituents while the antiserum employed as detector contained antibodies to a second.

Much of the egg albumin was excreted in the urine whereas the whole horse serum or the separate albumin and globulin fractions were not found in this fluid in an antigenically unchanged form except in a few of the animals and then only in low concentration. These observations confirm the earlier work of others (26, 27, 30).

The present experiments enable one to conclude that marked differences exist in the ease with which foreign proteins such as those employed, pass unchanged from the vascular system into the synovial fluid, aqueous humor, spinal fluid, and urine. The variation in permeability is determined not alone by the molecular size of the proteins but is dependent also on the anatomical and physiological differences in the barriers intervening between the blood capillaries and the body cavities studied. Of these barriers, aside from the kidney glomeruli (in the case of egg albumin), the synovial membrane and subsynovial tissues are the most permeable. This is in keeping with the anatomical fact that synovial membrane consists of several layers of mesenchymal cells separated in many instances from the rich subsynovial vascular system by only a few layers of cells (10). The greater permeability of the tissues separating the joint space from the vascular system may explain the higher total protein content of normal synovial fluid.

SUMMARY

1. Experiments designed to study, in the rabbit, the passage of foreign proteins from the blood stream into synovial fluid and to compare such passage with that taking place into the aqueous humor, spinal fluid, and urine are described.

2. Crystalline egg albumin and horse serum proteins regularly appeared in the knee joints within short periods of time following their intravenous injection.

3. These proteins also appeared promptly in the aqueous humor but in lower concentrations. In the spinal fluid they appeared only rarely and in minimal amounts.

4. Crystalline egg albumin was readily eliminated from the body *via* the urine. It was also removed rapidly from the knee joint and anterior chamber of the eye.

5. Horse serum proteins appeared only occasionally in the urine. Their concentration in the blood serum remained relatively high for several days.

Their increased concentration in the joint fluids in the longer experiments indicates that the rate of entrance exceeded the rate of removal.

6. Foreign proteins of the type employed were all found in the joint fluids in higher concentrations than they were in the other body fluids examined.

7. The possible significance of this study with respect to normal joint physiology and to certain abnormal joint conditions has been commented upon.

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