THE INFLUENCE OF INFLAMMATION ON THE ABSORPTION OF SUBSTANCES OF VARIED DIFFUSIBILITY*

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It has long been recognized that diffusible substances are absorbed from the tissues by direct entrance into the blood vessels. Dandy and Rowntree (1) made a quantitative study of absorption from the peritoneum. They found that when the diffusible dye, phenol red, is injected intraperitoneally, 40 to 60 per cent is excreted in the urine during the first hour after injection, while less than 0.1 per cent enters the thoracic lymph during this time. On the other hand, colloidal solutions and particles in suspension depend largely on the lymphatic system for absorption. Lewis (2) injected horse serum subcutaneously into a dog; using the complement fixation reaction, he was able to detect horse protein in the thoracic lymph 40 minutes after injection, while only after 3 hours did any protein appear in the blood. Drinker and Field (3) also found that horse serum is absorbed largely by way of the lymphatics. However, it should be mentioned that certain more diffusible colloidal solutions may enter the blood vessels directly. Bolton (4) found that the absorption of colloidal dyes of relatively small molecular dimensions is accomplished both by the vascular and by the lymphatic systems, but these colloidal dyes diffused into the blood more slowly than crystalloids.

Previous studies of the removal of substances from inflamed areas have dealt largely with the dissemination of bacteria. Noetzel (5) and Pawlowsky (6) found that when bacteria are injected into a joint they soon appear in the blood stream, but if the bacterial injections were made into a joint which had previously been treated by the injection of a sterile irritant, the dissemination of the bacteria was greatly inhibited. Hoehne (7) and Opie (8) found that if bacteria are injected into an inflamed peritoneal cavity, their entrance into the blood stream may be partially or completely inhibited.

Interest in the absorption of soluble substances from inflamed areas was aroused by the experiments of Opie on the Arthus phenomenon. Opie (9) demonstrated that when protein is injected into the skin of a specifically immunized animal, it remains at the site of injection, where contact of antigen and antibody produce an

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acute inflammatory reaction; and the injected protein does not enter the circulating blood. Menkin (10) extended these observations to a study of non-specific inflammation. He found that trypan blue, horse serum and colloidal iron are absorbed more slowly from inflamed than from normal areas. Okuneff (11) has presented corroborative data with trypan blue.

Few reports appear in the literature on the absorption of diffusible substances from inflamed areas. Underhill, Kapsinow and Fisk (12) concluded that phenol red was absorbed more slowly from burned areas than from normal areas. On the other hand Hudack and McMaster (13) found that inflammation facilitated the absorption of a diffusible as well as of an indiffusible dye. The object of the present experiments was to study the rate of absorption of soluble substances of varied diffusibility from inflamed areas.

General Methods

The substances used may be classified as proteins, carbohydrates and dyes. Horse serum globulin and crystalline egg albumin were the proteins studied. The carbohydrates were glucose and the pneumococcus Type I specific polysaccharide. The dyes were trypan blue, phenol red and brom phenol blue. These substances were chosen because they are relatively stable, non-toxic compounds, which can be detected readily by chemical or immunological methods. Furthermore, none of them (with the exception of several of the dyes) is known to enter into ready combination with protein under the conditions of the experiments. The effect of the combination of phenol red with protein is discussed under the experiments performed with that substance.

Production of Inflammation.—Male rabbits weighing 1600 to 2300 gm. were used. Animals of approximately the same weight group were used in each experiment. The inflammatory irritants employed were 5 per cent aleuronat and 3 per cent starch solution in 0.5 per cent saline, living avirulent staphylococcus cultures, staphylococcus vaccines, and in a few experiments broth which had been concentrated 20 times. The staphylococcus vaccines were prepared by heating 24 hour broth cultures of staphylococci at 60°C. for 30 minutes.

Great variation was found in the response of different animals to the same irritant. 24 hours after the injection of the irritant into the peritoneal cavity there was hyperemia and slight roughening of the peritoneal surfaces. No free fluid was present in the cavity. Shreds of fibrin were sometimes seen. Larger variation occurred in the subcutaneous inflammations. In general, these reactions were visible, 4 to 6 hours after the injection of the irritant, and consisted of swelling and induration of the skin, measuring about 4 to 7 cm. in diameter. On section there were marked edema and redness of the subcutaneous tissues, and to a lesser extent of the underlying muscle. Occasionally, a thin layer of pus

appeared under the skin at the end of 48 hours. Abscess formation occurred in only one experiment. The greatest variations were found in the cutaneous inflammations. The reaction to the staphylococcus injections was generally visible 2 to 6 hours after the injection, and consisted of a swollen, pale or erythematous area measuring about 1.5 to 4.0 cm. in diameter. Sometimes there was a hemorrhagic center surrounded by a pale area which in turn was surrounded by a zone of hyperemia. At other times a small necrotic focus appeared in the center of the injected area. The reactions produced by the injection of concentrated broth consisted of thickened erythematous areas measuring 1.5 to 2.5 cm. in diameter.

The Influence of Inflammation on the Absorption of Proteins

Methods.—Horse serum globulin was prepared by the addition of saturated ammonium sulfate to an equal quantity of horse serum. The resulting precipitate was centrifuged and dissolved in dilute sodium chloride solution. It was reprecipitated, and dialyzed against cold running tap water. No attempt was made to separate euglobulin from pseudoglobulin. Crystalline egg albumin was prepared by the method of Hopkins and Pincus (14). The egg albumin was recrystallized and dialyzed until the dialysate contained no further trace of ammonium sulfate. The protein solutions were sterilized by filtration through Berkefeld N or Seitz filters. The final dilution fluid contained 0.5 per cent salt solution. Protein content was determined by the method of Shevsky and Stafford (15) and was checked occasionally by Kjeldahl determinations.

Antisera were produced by the repeated intravenous and subcutaneous injections of the protein solution into rabbits. The titers obtained were 1–50,000 to 1–200,000 of globulin and 1–10,000 to 1–40,000 of egg albumin. Precipitin tests were performed by the usual antigen dilution method. 0.3 cc. of antiserum was added to 0.3 cc. of the diluted antigen. The tubes were allowed to stand at room temperature for 2 hours and then placed in the ice box overnight. Readings were made the following morning.

The attempt was made to determine if horse serum globulin disappeared from the site of inflammation in the skin. Rabbits were injected intracutaneously with an inflammatory irritant, for example, *Staphylococcus aureus*. The area of skin chosen was the flank about 4 cm. above the inguinal lymph node. 0.2 cc. of a 2 to 3 per cent globulin solution was injected into the inflamed area and into a similar control area on the opposite side. From 5 to 24 hours after the injection of the globulin, the animal was killed and the injected sites removed and weighed. The piece of skin 1.5 cm. in diameter was cut into fine pieces and ground in a mortar. Physiological saline solution was added to make a dilution of 1–10. The suspension was centrifuged and the supernatant used for precipitin tests. This method of extraction is evidently ineffective because the concentration of globulin showed scant change as the interval after injection increased. It is noteworthy that tests of the animals' sera following injection of globulin into inflamed or normal cutis were negative.

The Penetration of Horse Serum Globulin into the Serum after Injection into Normal and Inflamed Subcutaneous Tissue.—5 cc. of a 1–10 dilution of 24 hour broth culture of *Staphylococcus aureus* were injected subcutaneously into the flanks of rabbits. On the following day a thick, indurated area measuring about 4 to 6 cm. in diameter was seen. 2 cc. of a 3 per cent globulin solution were injected into this area in one group of animals 17 hours and in another 25 hours after the beginning of inflammation. A similar injection was made into a normal animal at the same time. The animals were bled from the ear vein at intervals varying from 5 to 24 hours after the injections of the globulin, and precipitin tests were performed on the sera.

Table I shows the results of tests made to determine the concentration of globulin in the blood serum at different intervals after injection into inflamed and into normal subcutaneous tissue. The three graphs of Fig. 1 are constructed from these tables and show the maximum titers at which globulin was demonstrable in the serum. The graphs of subsequent figures have been similarly constructed but tabulation of the data is omitted.

Graph 1 in Fig. 1 shows that the concentration of protein in the serum after simultaneous injection of inflammatory irritant and protein is for 24 hours almost the same as that following injection of protein alone. Inflammation that has lasted 17 or 25 hours (graphs 2 and 3 of Fig. 1) delays the penetration of globulin into the blood so that the concentration of protein in the serum at corresponding time intervals after injection of the protein is less than that of animals which received subcutaneous injections of globulin alone. 24 hours after the injection of the protein there is still slightly more horse serum globulin in the serum of the control animals than in that of the animals with inflammation. However, at this time there has been opportunity for considerable absorption of the protein from the inflamed areas.

The Penetration of Horse Serum Globulin into Serum after Injection into Inflamed Peritoneal Cavities.—5 cc. of a 1–10 dilution of 24 hour broth culture of *Staphylococcus aureus* were injected into the peritoneal cavities of rabbits. 1 to 2 cc. of a 3 per cent solution of horse serum globulin were injected simultaneously or on the following day. The same amounts of protein were also injected into normal rabbits. The rabbits were bled from the ear vein at stated intervals after the injection and precipitin tests were performed on the sera.

TABLE I

Presence of Horse Serum Globulin in the Blood Stream at Different Intervals Following Its Injection at the Site of Inflammation in the Subcutaneous Tissue Compared with That Following Injection into Normal Tissue

Time after injection of globulin	Dilutions					Dilutions				
	1:1	1:3	1:9	1:27	1:81	1:1	1:3	1:9	1:27	1:81
	After simultaneous injection of inflammatory irritant and globulin					After injection of globulin in normal skin				
hrs.										
5	+	tr.	0	0	0	+	sl. tr.	0	0	0
5	+	sl. tr.	0	0	0	+	" "	0	0	0
7	+	" "	0	0	0	+	" "	0	0	0
7	++	+	0	0	0	++	+	tr.	0	0
11	−	+	−	0	0	++	+	0	0	0
24	++	+	0	0	0	++	++	tr.	0	0
24	+++	++	+	0	0	++	+++	++	tr.	0
	With inflammation lasting 17 hrs.					After injection of globulin in normal skin				
5	sl. tr.	0	0	0	0	+	tr.	0	0	0
5	tr.	0	0	0	0	tr.	0	0	0	0
7	"	sl. tr.	0	0	0	−	+	+	sl. tr.	0
7	"	0	0	0	0	−	++	tr.	" "	0
11	−	+	sl. tr.	0	0	−	++	"	" "	0
24	++	+	" "	0	0	+++	++	+	" "	0
24	−	+	tr.	0	0	−	++	+	tr.	0
	With inflammation lasting 25 hrs.					After injection of globulin in normal skin				
5	0	0	0	0	0	++	+	0	0	0
5	0	0	0	0	0	0	0	0	0	0
7	tr.	0	0	0	0	++	+	tr.	sl. tr.	0
7	0	0	0	0	0	+++	++	+	tr.	0
11	++	sl. tr.	0	0	0	+++	++	+	sl. tr.	0
11	+	tr.	0	0	0					
24	+++	++	tr.	sl. tr.	0	+	++	+	tr.	0
24	+++	+	sl. tr.	0	0					

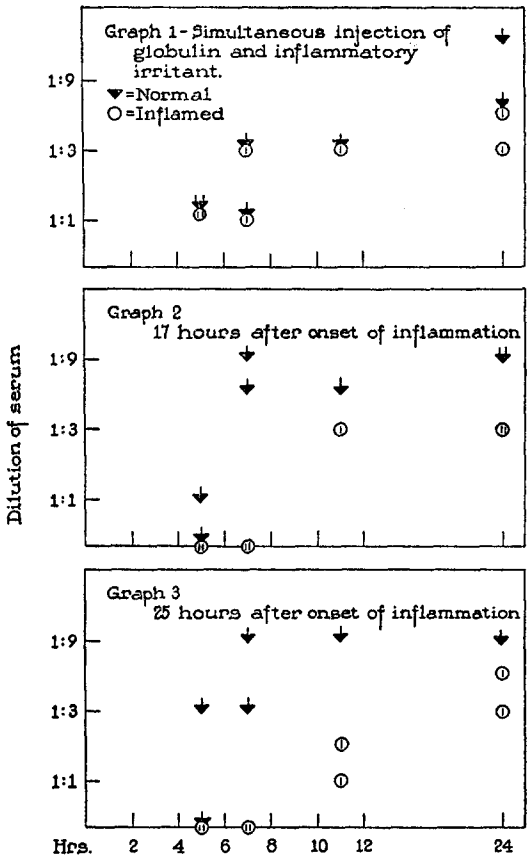

FIG. 1. The concentration of horse serum globulin in the circulating blood after its injection into normal and inflamed areas. The triangles in this and subsequent figures show the titer of globulin in the blood serum of rabbits after its injection into normal subcutaneous tissue, and the circles show its concentration after injection into inflamed subcutaneous tissue. The short lines above the triangles or within the circles indicate the number of observations at a given point. Graph 1 shows the concentration of horse serum globulin in the blood of rabbits after simultaneous injection of globulin and the inflammatory irritant compared with its concentration at corresponding intervals after injection of globulin alone. Graph 2 shows the concentration of globulin in the blood serum of animals that have received globulin 17 hours after the onset of inflammation compared with the corresponding controls. Graph 3 shows the concentration in the blood serum of rabbits that received globulin 24 hours after the onset of inflammation.

Fig. 2 shows that the penetration of horse serum globulin into the serum of the animals with the inflamed peritoneal cavities is delayed,

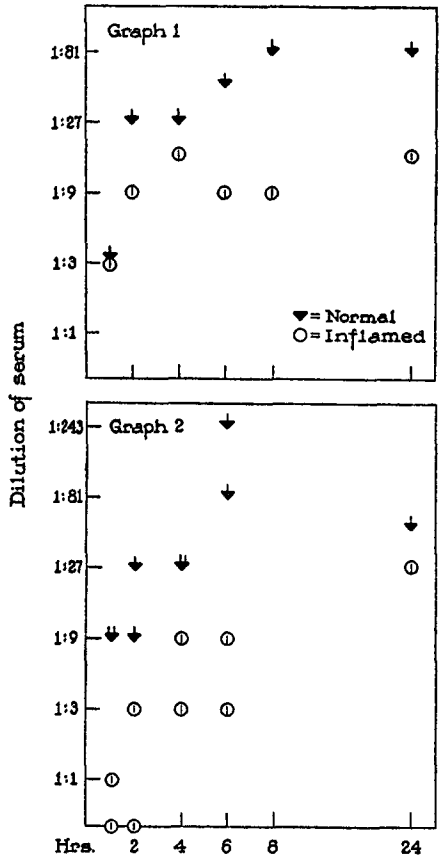

FIG. 2. The concentration of horse serum globulin in the circulating blood after its injection into normal and inflamed peritoneal cavities; graph 1, with simultaneous injection of globulin and inflammatory irritant, and graph 2, with injection of globulin 18 hours after onset of peritonitis, in both instances compared with controls.

even when the irritant is injected simultaneously with the protein (graph 1), but retardation of absorption is greater when the injection of globulin is made 18 hours after the onset of inflammation (graph 2).

The Penetration of Crystalline Egg Albumin into the Serum after Injection into Normal and Inflamed Subcutaneous Tissues.—Rabbits were injected subcutaneously with 3 cc. of staphylococcus vaccine or 2 cc. of a 1–10 dilution of a 24 hour broth culture of *Staphylococcus aureus*. On the following day, 1 or 2 cc. of 3 per cent egg albumin were injected into the subcutaneous tissue of normal animals. The rabbits were bled at intervals and precipitin tests were performed on the sera.

The results are given in Fig. 3. There is a slightly higher concentration of egg albumin in the serum of the normal animals than in the animals with inflamed subcutaneous tissue. This difference was most apparent 2 to 4 hours after injection. 24 hours after injection most of the egg albumin had left the sera of both groups of animals.

The Penetration of Crystalline Egg Albumin into the Serum after Injection into Normal and Inflamed Peritoneal Cavities.—Rabbits were injected intraperitoneally

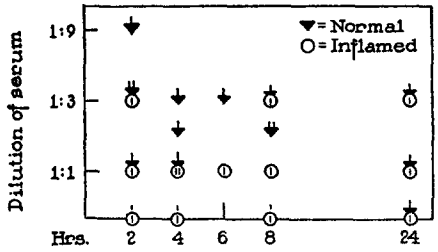

FIG. 3. The concentration of crystalline egg albumin in the circulating blood after its injection into the normal subcutaneous tissue and into the inflamed subcutaneous tissue from 19 to 27 hours after the onset of inflammation.

with 5 to 10 cc. of a 24 hour staphylococcus culture, diluted 1–10, or with 10 cc. of aleuronat starch mixture. Simultaneously, or on the following day, 2 to 8 cc. of 5 per cent egg albumin solution were injected intraperitoneally into these animals, and into normal rabbits. Blood was drawn at intervals and the sera were used for precipitin tests.

The results are given in Fig. 4. Following the simultaneous injection of inflammatory irritant and crystalline egg albumin the concentration of the latter in the blood serum differs little from that of animals that received albumin alone (graph 1). 2 hours after intraperitoneal administration of crystalline egg albumin from 18 to 22 hours after onset of inflammation (graph 2) there was more egg albumin in the sera than in those of the normal animals that received crystalline egg albumin. This difference was still apparent 4 to 6 hours

after injection. By 24 hours most of the egg albumin had disappeared from the sera of both groups of animals.

In a few experiments urine from the injected animals was tested for the presence of egg albumin. It was found that the protein could be detected in the urine as early as 2 hours after injection. The amount of protein excreted seemed to have no constant relationship to the amount in the circulating blood.

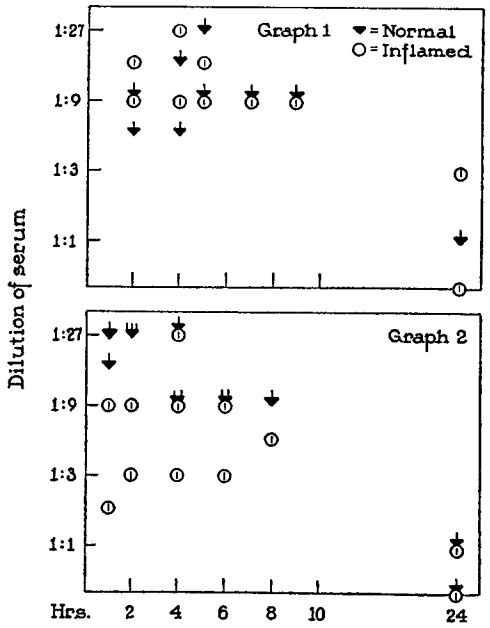

FIG. 4. The concentration of crystalline egg albumin in the circulating blood after its injection into the normal and inflamed peritoneal cavity; graph 1, with simultaneous injection of crystalline egg albumin and inflammatory irritant, and graph 2, with injection of globulin from 18 to 22 hours after onset of peritonitis, in both instances compared with controls.

The Influence of Inflammation on the Absorption of Carbohydrates

The pneumococcus Type I specific polysaccharide[1] and glucose were used in these experiments. The polysaccharide had been prepared according to the method of Heidelberger, Kendall and Scherp (16).

[1] The polysaccharide was obtained through the courtesy of Dr. Kenneth Goodner and Dr. Frank Horsfall of The Rockefeller Institute for Medical Research.

Earlier information (Heidelberger, 17) has indicated that the pneumococcus type specific carbohydrates are diffusible compounds of low molecular weight. However, the polysaccharides prepared by the newer methods, which ensure against their hydrolysis, appear to be more indiffusible compounds. Thus, the recent work of Avery and Goebel (18) demonstrates that 7 days after the intravenous injection of 17 mg. of pneumococcus Type I polysaccharide into rabbits, this substance may still be detected in the circulating blood. Babers and Goebel (19) have studied the rate of diffusion of the pneumococcus Type III polysaccharide and have concluded that it is an extremely indiffusible compound, the molecular weight of which they calculate to be 118,000.

Antisera against the polysaccharide were prepared by the repeated intravenous injection of a Type I pneumococcus vaccine into rabbits. A smooth organism which had recently been passed through mice was used in the preparation of the vaccine. The titer obtained was between 1–1,000,000 and 1–5,000,000.

A 0.1 per cent solution of the carbohydrate was prepared by dilution with physiological saline solution. It was sterilized by heating for 1 hour at 56°C. Preliminary experiments were made to determine the diffusibility of the compound in the animal body. In the preliminary experiments, 2.5 mg. and 5.0 mg. respectively of the Type I carbohydrate were injected intravenously into rabbits. The animals were bled from the ear vein and the sera used for precipitin tests.

It was found that the specific polysaccharide titer of the blood dropped somewhat in the first 10 minutes after intravenous injection. Thereafter the titer remained approximately constant for 24 hours, and small amounts of the substance were still present in the circulating blood 72 hours after injection. Very small amounts of the polysaccharide appeared in the urine during the first day after injection.

The Penetration of Pneumococcus Type I Polysaccharide into the Serum after Injection into Normal and Inflamed Peritoneal Cavities.—5 mg. of pneumococcus Type I polysaccharide in 5 cc. of physiological saline solution were injected intraperitoneally into normal rabbits and into those which had received 5 cc. of a 1–10 dilution of staphylococcus culture intraperitoneally on the previous day.

The results are given in Fig. 5. There is a consistent difference between the penetration of the pneumococcus Type I polysaccharide into the serum from normal and into that from inflamed peritoneal cavities. 2 hours after injection more carbohydrate is present in the blood of the normal than of the inflamed animal. This difference is still apparent although to a lesser extent 24 hours after injection.

The Disappearance of Glucose after Injection into Normal and Inflamed Peritoneal Cavities.—5 cc. of *Staphylococcus aureus* vaccine were injected intraperitoneally into rabbits. On the following day 10 to 100 cc. of 5 per cent glucose solution were injected intraperitoneally into the treated rabbits and into normal animals. The animals were killed by air injection 30 minutes to 60 minutes after the injection of the glucose. In the rabbits that received 10 to 20 cc. of glucose, 100 cc. of saline solution were introduced into the peritoneal cavity immediately after death. The abdominal cavity was then opened and the contents allowed to drain into a large funnel. An additional 100 cc. of saline were used to wash out the peritoneal cavity. When 100 cc. of glucose had been injected, the abdominal cavity was opened immediately after death, and the contents allowed to drain into a funnel. The remaining procedure was the same. Glucose was determined by the method of Miller and Van Slyke (20).

Table II shows that the absorption of glucose from the inflamed peritoneal cavity proceeds as rapidly as absorption from the normal

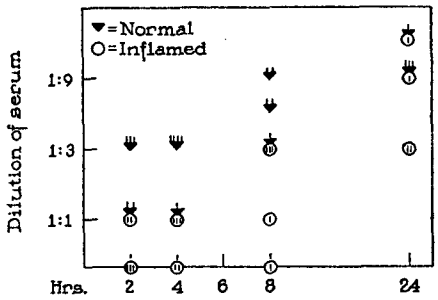

FIG. 5. The concentration of the specific polysaccharide of pneumococcus Type I in the circulating blood after its injection into the normal peritoneal cavity and into the inflamed peritoneal cavity from 13 to 20 hours after the onset of peritonitis.

peritoneal cavity. When large amounts of glucose are injected (5100 mg.), there may be more rapid absorption from the inflamed than from the normal peritoneal cavity.

The Influence of Inflammation on the Absorption of Dyes

The Disappearance of an Indiffusible Dye from the Inflamed Cutis.—A 1 per cent solution in 0.5 per cent saline of trypan blue was used. 0.05 to 0.1 cc. of the solution was injected into the skin of rabbits into which 0.2 cc. of a 24 hour broth culture of *Staphylococcus aureus* had previously been injected. The sites chosen were the anterior surface of the tip of the ear or the abdominal wall. The contralateral normal side was used as a control. The spread of the dye was observed at varying intervals and recorded by means of colored drawings.

Trypan blue (Table III) remained localized within a small area of the inflamed skin, whereas a wide diffuse coloration appeared in the

TABLE II

Absorption of Glucose after Injection into the Peritoneal Cavity

Experiment No.	Weight	Amount injected	Time glucose remained in body	Amount recovered	Absorbed
	gm.	*mg.*	*min.*	*mg.*	*per cent*
	Animals with Inflamed Peritoneal Cavities				
1	1835	525	32	113.3	78.5
2	1700	1000	33	365.0	63.5
3	1950	1000	35	474.0	52.6
4	1680	1000	30	698.0	30.2
5	1650	5100	60	2005.0	60.6
6	1690	5100	60	1985.0	61.0
	Animals with Normal Peritoneal Cavities				
1	1915	525	32	203.9	61.1
2*	1725	1000	40	335.0	66.5
3	1750	1000	35	420.0	58.0
5	1630	5100	60	2550.0	48.2
6	1680	5100	60	2475.0	51.4

* Cysticerci of *Taenia pisiformis* were present in the abdominal cavity and upon the omentum and liver.

TABLE III

Spread of Trypan Blue Injected into Normal and Inflamed Intracutaneous Sites

Experiment No.	Interval after injection of dye	Colored area in normal site	Colored area in inflamed site	Difference in color	Comment
	hrs.	*sq. mm.*	*sq. mm.*		
1	8	300	28	Inflamed darker	
2	24	392	32	" "	2 days later inflamed site is colorless, normal measures 306 sq. mm.
3	24	150	48	—	2 days later inflamed site measures 12 sq. mm., normal 338 sq. mm.
4	24	450	90	—	2 days later inflamed site measures 48 sq. mm., normal 576 sq. mm.

normal skin. Nevertheless, the ultimate disappearance of the dye was sometimes effected more rapidly from the inflamed than from the normal skin. This occurred several days after injection.

The Disappearance of Brom Phenol Blue from the Inflamed Cutis.—0.2 to 0.5 cc. of staphylococcus culture was used to produce the inflammatory reaction in the skin of rabbits. The sites chosen were the anterior abdominal wall about 4 cm. from the midline, and the skin of the anterior surface of the tip of the ear. 18 to 24 hours after the injection of the irritant, 0.05 or 0.1 cc. of brom phenol blue (0.2 to 0.4 per cent solution in physiological saline) was injected into the inflamed site

TABLE IV

Disappearance of Brom Phenol Blue after Injection into Normal and Inflamed Cutaneous Areas

Experiment No.	Amount of dye injected	Time from injection of dye to last observation	Colored area remaining in		Color difference
			Inflamed site	Normal site	
		hrs.	*sq. mm.*	*sq. mm.*	
1	0.05 cc. 1–10	3	9	42	Normal site lighter
2	0.05 " 1–10	3	2	16	None
3	0.05 " 1–10	3	14*	80	"
4	0.05 " 1–10	3	4	156	"
5	0.05 " 1–20	3	0	6	—
6	0.05 " 1–40	2	0*	20	—
7	0.05 " 1–40	2	0	16	—
8	0.05 " 1–10	7	80	70	Inflamed site much lighter
9	0.05 " 1–10	7	72	120	Inflamed site lighter
10	0.05 " 1–10	7	64	143	Normal site lighter
11	0.05 " 1–10	7	48	90	None
12	0.05 " 1–10	4	56	30	Inflamed site lighter
13	0.05 " 1–10	7	12	42	" " "
14	0.05 " 1–20	7	2†	0	—
15	0.05 " 1–20	7	1†	0	—
16	0.05 " 1–20	7	6†	0	—
17	0.1 " 1–120	24	4†	0	—
18	0.1 " 1–20	24	1†	0	—

* 48 hour inflammation.

† Necrosis.

and into the contralateral normal side. After the wheal caused by the injection had disappeared, the dye-stained areas were measured and recorded by means of colored drawings. Further drawings were made at intervals until the dye had disappeared in one or both of the injected sites. When the observations were not continued until the disappearance of the dye, the last reading is given.

The results are listed in Table IV. This table shows that inflammation facilitates the disappearance of this dye if the inflammatory

process does not proceed to necrosis. When necrosis appears the dye becomes firmly fixed in the necrotic tissue.

The Disappearance of Phenol Red from the Inflamed Cutis.—Sterile ampoules of phenol red, containing 6 mg. per cc.,[2] were used. 0.2 to 0.3 cc. of the dye was injected intracutaneously into normal areas and into those which had previously been treated by the injection of 0.1 to 0.3 cc. of *Staphylococcus aureus* cultures. At stated intervals the animals were killed and the injected areas, measuring 1.5 cm. in diameter, were removed. The tissues were weighed and cut into fine strips. They were then ground in a mortar and repeatedly extracted with 95 per cent

TABLE V

Extraction of Phenol Red from Normal and Inflamed Cutaneous Areas

Experiment No.	Duration of inflammation before injection of dye		Time dye remained in body	Amount injected	Amount recovered from	
					Inflamed site	Normal site
	hrs.	*min.*	*min.*	*mg.*	*mg.*	*mg.*
1	1	00	15	1.2	0.18	0.38
2	1	00	15	1.2	0.28	0.35
3	1	00	30	1.2	0.08	0.30
4	3	00	30	1.2	0.28	0.19
5	3	00	30	1.2	0.22	0.11
6	22	15	30	1.8	1.5*	0.44
7	22	00	40	1.2	0.23	0.24
8	22	00	40	1.2	0.11	0.30
9	17	15	45	1.2	0.22	
10	17	15	45	1.2	0.19	0.098
11	3	00	50	1.8	0.52	0.39
12	18	00	50	1.8	0.60	0.41

* Marked necrosis.

alcohol, until the extracts showed no further pink color on the addition of alkali. Colorimetric determinations of the phenol red content of the extracted fluid were made. This method had previously been found satisfactory by Marshall and Vickers (21).

The results are given in Table V. Great variations occur in the disappearance of phenol red from the inflamed cutaneous areas. In the first 15 minutes after injection the dye appears to be absorbed more rapidly from the inflamed site. In the later stages of absorption no difference can be found between the disappearance of the dye from

[2] Prepared by Hynson, Westcott and Dunning.

the normal and from the inflamed areas. However, in examining these results the familiar observation that phenol red readily combines with plasma proteins must be taken into consideration. It was found by Marshall and Vickers (21) that in the rabbit 90 to 98 per cent of phenol red may be bound to plasma proteins. The effect of the combination would not be apparent when the dye is present in excess; but when inflammation is far advanced or only a small amount of dye is present, as in the late stages of absorption, there is presumably an excess of protein over dye and combination between them probably occurs.

The Excretion of Phenol Red in the Urine after Injection into Normal and Inflamed Subcutaneous Tissue.—1 cc. of phenol red was injected into normal and inflamed subcutaneous tissues of male rabbits weighing 1800 to 2300 gm. Inflammation was produced by the injection of 3 cc. of a 1–10 dilution of a 24 hour staphylococcus culture or 5 cc. of an aleuronat starch mixture. The animals were given 100 cc. of water by stomach tube about 1 hour before the injection of the dye. Another 50 cc. of water was given 1 to 2 hours later. Urine was obtained by catheterization. In some instances numerous difficulties were experienced in obtaining suitable specimens; it was found that animals developed anuria for several hours after the catheterization was attempted and it was necessary to wait until a free flow of urine occurred before starting the experiment. After each catheterization the bladder was washed with 10 cc. of water to ensure complete collection of the urine. Quantitative colorimetric estimations of the amount of phenol red were made according to the method of Rowntree and Geraghty (22).

The results of the experiments are given in Table VI which shows the average percentage excretion of the dye at a given time as well as the total excretion. The results in the two groups of animals are practically identical. They indicate that there is rapid excretion of phenol red after its injection into both normal and inflamed subcutaneous tissues.

The Excretion of Phenol Red in the Urine after Injection into Normal and Inflamed Peritoneal Cavities.—The procedures were identical with those given above, except that the dye was injected into the peritoneal cavities instead of the subcutaneous tissues. Inflammation was produced by the injection of 5 cc. of a 1–10 dilution of staphylococcus culture or 10 cc. of aleuronat starch mixture. Phenol red (6 mg.) was introduced into the peritoneal cavity from 18 to 24 hours after the injection of the irritant.

TABLE VI

Percentage Excretion of Phenol Red in Urine after Subcutaneous Injection of 6 Mg.

Experiment No.	Time (hours)					
	½	1	2	3	4	Total
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	Animals with Inflamed Subcutaneous Tissues					
1	17.5	25.0	15.0	12.5	1.7	71.7
2	12.6	20.0	24.2	12.0	5.9	74.7
3	26.8	26.6	18.5	5.0	0.6	77.5
4	11.1	22.9	29.4	14.2	5.0	82.6
5	25.0	25.5	14.1	—	5.6	70.5
Average...	18.6	24.0	20.3	10.9	3.7	77.6
	Animals with Normal Subcutaneous Tissues					
1	25.0	22.5	12.3	5.0	1.5	66.5
2	20.0	21.0	29.7	3.6	5.6	79.9
3	14.1	25.0	23.8	8.3	7.1	78.3
4	14.1	36.7	8.4	14.9	6.1	80.2
Average...	18.3	26.3	18.6	8.0	5.0	76.2

TABLE VII

Percentage Excretion of Phenol Red in Urine after Intraperitoneal Injection

Experiment No.	Time (hours)				
	½	1	2	4	Total
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Animals with Inflamed Peritoneal Cavities				
1	15.7	24.5	20.0	17.6	76.8
2	22.7	19.5	18.1	9.3	69.6
3	17.0	20.8	23.0	7.3	68.1
4	23.3	18.8	9.9	2.6	54.6
Average....	19.7	22.15	17.8	9.2	68.8
	Animals with Normal Peritoneal Cavities				
1	21.4	28.6	15.6	13.0	78.6
2	21.0	26.8	16.6	7.2	71.6
3	9.5	23.5	14.4	11.3	58.7
4	16.6	24.3	15.6	5.6	62.1
Average....	17.1	25.8	15.5	9.2	67.6

The results are given in Table VII. No differences are seen between absorption of the dye from normal and inflamed peritoneal cavities, as indicated by the rate of its excretion in the urine.

The Disappearance of Phenol Red after Injection into Normal and Inflamed Subcutaneous Tissues.—5 cc. of a 1–10 dilution of a 24 hour broth culture of *Staphylococcus aureus* or 5 cc. of aleuronat starch mixture were injected subcutaneously into the dorsal surface of the thighs of rabbits. On the following day

TABLE VIII

Phenol Red Recovered from the Site of Inflammation after Subcutaneous Injection of 6 Mg.

Experiment No.	Duration of inflammation before injection of dye		Time dye remained in body	From inflamed site	From normal site
	hrs.	*min.*	*min.*	*mg.*	*mg.*
	0	00	Excised immediately		5.58
	0	00	" "		3.3 (3.6 injected)
1	25	45	10	4.8	5.2
2	24	00	15	3.6	4.0
3	24	00	15	3.6	3.6
4	14	00	16	3.2	3.4
5	16	00	20	1.6	2.1
6	24	45	25	3.0*	2.2
7	1	00	25	1.2	2.4
8	21	00	30	1.8	2.4
9	24	20	35	1.3	1.6
10	21	30	40	0.88	2.5
11	22	20	50	0.93	1.7
12	25	00	110	0.55	0.35

* Abscess formation.

1 cc. (6 mg.) of phenol red was injected into the inflamed area and into the contralateral normal area. At stated intervals the rabbits were killed by air injection and a circular piece of skin measuring 4 cm. in diameter, the center of which corresponded with the site of injection, was removed. The underlying fascia containing dye was also excised. Extraction with 95 per cent alcohol was performed by the procedure used after intracutaneous injection. The amount of dye extracted was determined colorimetrically.

The results are shown in Table VIII. In all but two of the experiments the phenol red has disappeared more rapidly from the inflamed

than from normal subcutaneous tissue. In one instance an abscess had formed and in the other about 2 hours had elapsed between injection of the dye and its extraction.

DISCUSSION

The substances used in these experiments fall into the general categories of proteins, carbohydrates and dyes. Of the proteins, serum globulin is a very slowly diffusible compound with a molecular weight of approximately 140,000 (see Cohn, 23). Egg albumin is composed of smaller, more diffusible molecules weighing 34,500 (Svedberg, 24).

Several investigators (2, 3), have demonstrated that the parenteral absorption of proteins is accomplished chiefly by the lymphatic system. Nevertheless, a certain portion of injected protein may enter the blood vessels directly (3). This portion is probably larger in the case of the more diffusible egg albumin than in the case of globulin. In our experiments differences in diffusibility of the two proteins are indicated by the fact that egg albumin reaches a maximum concentration in the blood about 2 to 4 hours after intraperitoneal injection. Thereafter, the concentration in the blood falls sharply, and large amounts of the albumin appear in the urine. On the contrary, the maximum concentration of globulin in the blood is attained only 24 hours after intraperitoneal injection, and the excretion of this protein is very slow.

When crystalline egg albumin or horse serum globulin is injected into inflamed subcutaneous areas or inflamed peritoneal cavities, its absorption is definitely retarded. This retardation is more conspicuous in the case of globulin than of egg albumin.

A further difference in the behavior of these proteins is seen when they are injected simultaneously with the irritant. Under these circumstances, concentration of egg albumin in the serum of the animal with the inflamed peritoneal cavity is higher than the concentration in the serum of the normal animal. The difference is apparent 2 hours after the injection. This observation appears to parallel the experiments of Okuneff (25) who found that absorption of trypan blue from the subcutaneous tissue is accelerated when this dye is injected simultaneously with an irritant. Hoehne (7) has shown that the

absorption of bacteria is similarly accelerated in the early stages of inflammation.

Data showing the effect of inflammation on the absorption of two carbohydrates have been given. On the one hand, the pneumococcus Type I specific polysaccharide is prevented from leaving the inflamed peritoneal cavity. On the other, the absorption of glucose is unaffected or accelerated by inflammation. These compounds differ sharply in diffusibility. The pneumococcus polysaccharide is an exceedingly indiffusible substance, as demonstrated by the difficulty with which it leaves the blood after intravenous injection. It is likely that, in common with other indiffusible substances, it is absorbed chiefly by way of the lymphatic system (3).

The extreme diffusibility of glucose is a familiar property of this substance. It is absorbed readily after parenteral injection and undoubtedly enters the blood vessels directly. Inflammation in no way deters the absorption of this carbohydrate. The difference in the rate of absorption of the two carbohydrates in the presence of inflammation is apparently related to differences in their diffusibility.

Similarly, the effect of inflammation on the absorption of dyes depends largely on the diffusibility of the compounds. Phenol red (phenolsulfonphthalein) is the most diffusible of the dyes studied. The excretion of this dye in the urine after injection into subcutaneous tissue or the peritoneal cavity indicates that there is no difference between its absorption from normal and from inflamed areas. However, direct extraction of phenol red from the tissues clearly demonstrates an acceleration in the absorption of this dye from inflamed subcutaneous areas. The method of direct extraction is undoubtedly a more accurate index of absorption than is the estimation of excretion. The latter is subject to several experimental errors, such as those that occur in collection of urine samples. It introduces also the factor of renal efficiency, which differs widely in different animals.

Phenol red has the property of entering a chemical or physical union with plasma proteins when these are present in excess (Marshall and Vickers, 21). Because of this property, the diffusible dye may readily be converted into a relatively non-diffusible compound. Furthermore, the ratio of combined to uncombined dye will vary with the amount of protein present, the pH of the solution, etc. These

considerations make it difficult to estimate differences in the absorption of unaltered phenol red from normal and inflamed cutis, where the amount of dye injected is small and protein may be present in excess.

The diffusible dye brom phenol blue is absorbed more rapidly from inflamed than from normal skin. However, when necrosis occurs in the inflamed area, it inhibits the absorption of the dye. Where there is necrosis, inflammation, which can occur only in living tissues, ceases. Thrombosis of blood and lymphatic vessels and the formation of a fibrous wall around the necrotic area sharply delimit the dead from the living tissue. Conspicuous inhibition of absorption was noted also in several experiments in which phenol red was injected into necrotic tissue.

Trypan blue shows little tendency to spread in the inflamed skin in contrast to the wide, diffuse coloration which appears when the dye is injected into normal skin. Even though the dye ultimately disappears from the inflamed site more rapidly than from the normal one, it remains localized in a relatively small area throughout the period of disappearance.

The adsorptive properties of many dyes raise some objection to their use. Okuneff (25) has shown that charcoal, bolus alba and proteins adsorb trypan blue: the dye is partially decolorized. Despite this fact he concluded (11), as had Menkin, that inflammation definitely retards the absorption of this dye. In the present experiments the fact that the trypan blue remained darker in color in the inflamed than in the normal site argues against any considerable adsorption by the protein in the inflamed tissue.

In general, these experiments indicate that inflammation inhibits the absorption of slowly diffusible compounds and accelerates the absorption of readily diffusible ones.

The acceleration of absorption of diffusible compounds in the inflamed area is undoubtedly related to the rapid flow of blood since these compounds enter the blood vessels directly. The mechanism of the inhibition of absorption of the relatively indiffusible materials is less clear. The process may be one that concerns the lymphatics, for the substances affected by it depend largely on the lymphatic system for absorption. In this connection Menkin (10)

has suggested that the presence of fibrin in the tissue spaces and lymphatic vessels prevents the entrance of substance into the latter.

The agents that produce inflammation are commonly of a bacterial or parasitic nature. A large body of experimental data (6–9) leaves no doubt that bacteria are inhibited or prevented from leaving the inflamed area, within which the microorganisms tend to be dissolved or destroyed. During this process, the fate of toxic bacterial products is of the utmost importance, for these may offer greater potential dangers than the bacteria themselves. Toxins and other primary bacterial derivatives are soluble, whereas the bacterial body is particulate; they may readily gain access to vital organs by way of the blood. Localization of these products in the inflamed area, which is well prepared to destroy and digest them, may prevent widespread bodily injury.

The inhibiting mechanism evidently does not operate with equal facility upon all substances. The absorption of globulin from the inflamed area is retarded more readily than the absorption of egg albumin, but complete inhibition of the absorption of soluble substances has not been observed. On the contrary, several investigators have reported that inflammation may completely prevent the entrance of bacteria into the blood. Thus, Opie (9) found that the penetration of streptococci from the inflamed peritoneal cavity into the blood might be completely prevented. Nevertheless, some of the animals in which sterile blood cultures were obtained for hours after injection of the bacteria, showed symptoms of toxemia before a terminal bacteremia was observed.

It appears probable that the degree of inhibition of absorption from the inflamed area is related to the diffusibility of the compound studied; greater indiffusibility is accompanied by greater inhibition of absorption. Bacteria are localized more effectively than their derivatives, among which there are probably further differences according to diffusibility.

In order that the inflammatory process may pursue a favorable course it is important that both the injurious and the defensive substances be retained within it. Mobilization of such substances in the inflamed site is well recognized. "The inflammatory reaction affords means by which various substances, notably enzymes, are

delivered in unusual quantity in response to unusual local need" (Opie, 26). It is notable that antibodies and many known enzymes are proteins and therefore relatively indiffusible compounds. They, together with indiffusible bacterial and tissue products, are retained in the inflamed site where interaction between them occurs.

On the contrary, it is important that end products of enzymatic digestion and other diffusible metabolites produced in the inflamed site should not accumulate there. Such accumulation would tend to add further injury to an already injured tissue. Our results indicate that rapid absorption of these substances from the inflamed site occurs: upon entrance to the blood they are readily excreted.

CONCLUSIONS

1. Inflammation retards the absorption of horse serum globulin and crystalline egg albumin from the peritoneal cavity and subcutaneous tissue, but retardation of the absorption of crystalline egg albumin is less than that of globulin, which is less diffusible.

2. Inflammation retards the absorption of the specific polysaccharide of pneumococcus Type I from the peritoneal cavity; inflammation may accelerate, but does not hinder, the absorption of glucose from the peritoneal cavity.

3. Inflammation retards the spread of trypan blue in the skin, but accelerates absorption from the skin of the more diffusible dye, brom phenol blue.

4. Phenol red is excreted in the urine with equal rapidity after injection into normal and into inflamed subcutaneous tissue or into normal and into inflamed peritoneal cavities. Direct extractions of phenol red from inflamed subcutaneous sites indicate that inflammation accelerates the absorption of the dye from these areas.

5. Inflammation retards the absorption of the indiffusible proteins, carbohydrates and dyes; it tends to accelerate the absorption of the diffusible carbohydrates and dyes.

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