no increase is observed upon treatment with a heterologous serum at the dilution used.

Further, the investigation of the screening action of films transferred on the slide on top of the antigen films brought out most surprising results, which are, in fact, the main object of this note. It can be seen from the data in Tables 2 and 3 that screens consisting of stearic acid or protein layers do not prevent considerable specific adsorption of antibodies. When the antigen films consist of three double layers of bovine albumin, a specific reaction still occurs in spite of a screen of four double layers of stearic acid. Analogous results were obtained with screens of octadecylamine and mixed screens of octadecylamine and stearic acid.

TABLE 2

SCREENING EFFECT OF STEARIC ACID LAYERS ON THE ANTIGEN-ANTIBODY REACTION

	Antigen films: 3 double layers of bovine albumin (50A)		Antigen films: 1 double layer of egg albumin (16A)	
Screen: Number of stearic acid layers	Increase in A units after adsorption of anti-bovine albumin serum	Increase in A units after adsorption of anti-egg albumin serum	Increase in A units after adsorption of anti-egg albumin serum	Increase in A units after adsorption of anti-bovine albumin serum
2	87	0	18	0
4	61	2	13	5
6	36	6	12	10
8	29	13		

TABLE 3

SCREENING EFFECT OF EGG ALBUMIN LAYERS ON BOVINE ALBUMIN-ANTIBOVINE ALBUMIN REACTION

Antigen: Number of bovine albumin layers	2	2	2	2	2	6	6
Screen: Number of egg albumin layers	2	4	6	8	10	6	10
Increase in A units after adsorption with antibovine albumin serum	41	28	17	9	0	86	58

The interpretation of these results now becomes important. Perhaps the most obvious explanation is that stearic acid or protein films have holes through which the antibody molecules can reach the antigenic film. Such an explanation, however, is scarcely satisfactory if one considers the closely packed structure of stearic films in relation to the size of the antibody molecule (molecular weight $\simeq$ 160,000). Furthermore, an extensive series of experiments carried out in this laboratory have shown that stearic acid films may act as a perfect screen for certain types of reaction. For instance, at the proper pH a layer of insulin molecules 150A thick[5] (molecular weight $\simeq$ 40,000) can be adsorbed on a slide covered by a layer of protamine. Nevertheless, a monolayer of stearic acid deposited on the protamine prevents subsequent adsorption of insulin molecules.

The following explanation of our results is offered tentatively. It is assumed that the effective range of action between a film of antigen and an antibody molecule might extend to an order of hundreds of A instead of a few A as in the case of forces between individual atoms. In order to achieve such a long range, it is considered possible that a field of force of greater magnitude might result from an integrated action of the many elementary units which build up the single large molecules of antigen and antibody in an orderly way. It is a matter of speculation how this field is realized, but it is not impossible that it could result along the lines indicated by London.[6] Under favorable conditions, the field of force of the antigen film could be enhanced as the number of layers is increased, which seems to occur in the case of bovine albumin films. The data reported above may thus be interpreted in the sense that no direct contact is necessary between a film of antigen and the corresponding antibodies to demonstrate a specific interaction.

If this interpretation is correct, it means that in the animal body the sphere of action of antibodies is much greater than commonly supposed. An interaction might even occur through a thin biological membrane.

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METABOLISM IN DIABETIC COMA PRODUCED BY ALLOXAN[1]

IT has been observed that the injection of alloxan in rats will produce varying degrees of diabetes mellitus—even severe coma—which is similar to that seen in human beings.[2,3] Alloxan has, therefore, provided us with a valuable experimental tool, with which to study the metabolism in diabetic coma and the results of therapy.

In human diabetic coma there is a marked elevation in plasma inorganic phosphate which decreases rapidly, following administration of insulin.[4,5] The mechanism of this fall is still obscure. Studies on normal animals show that insulin causes a rise in acid-

[5] G. H. A. Clowes, "Recent Advances in Surface Chemistry and Chemical Physics," The American Association for the Advancement of Science, No. 7, p. 61. 1939.

[6] F. London, "Surface Chemistry," The American Association for the Advancement of Science, No. 21, p. 141. 1943.

[1] These studies were aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] J. S. Dunn and N. G. B. McLetchie, *Lancet*, 245: 384, 1943.

[3] R. E. Janes and C. E. Friedgood, *Endocrinology*, 36: 62, 1945.

[4] G. M. Guest and S. Rapoport, *Am. Jour. Dis. of Children*, 58: 1072, 1939.

[5] M. Franks, R. F. Berris, N. O. Kaplan and G. B. Myers. In preparation.

soluble phosphates in the liver.[6,7,8] The present investigation was undertaken to examine the relationship between plasma inorganic phosphate and the acid soluble phosphates of the liver in diabetic coma.

Long-Evans hooded male rats of 200 to 300 grams in weight were used. The experimental animals were fed an equal amount of a stock diet for one week prior to the time of injection with alloxan. Control animals were similarly fed for one week before sacrificing.

Alloxan was injected intraperitoneally in a single dose of 200 mgm per kilo of body weight. Following the administration of alloxan, all animals consumed the same quantity of the stock diet. Urines were tested daily for sugar and ketone bodies. Only those animals which were clinically in diabetic coma were used. Each animal exhibited Kussmaul respiration, was semi-conscious or totally unconscious, and the urine showed a four-plus sugar (5–10 per cent.) and a four-plus acetone. Those animals which attained this stage of diabetes between 70 to 80 hours after the injection of alloxan were employed, for reasons which will be described in subsequent reports.

All animals were fasted for a period of 16 hours, before they were sacrificed. Under sodium pentobarbital anesthesia, blood samples were drawn from the inferior vena cava, and a portion of the liver was immediately removed and rapidly frozen. Blood samples were then analyzed for sugar and inorganic phosphate and the liver was analyzed for glycogen and acid-soluble phosphates.

Table 1 includes values for normal animals, "alloxan refractive" animals and severe untreated comas. The term "alloxan refractive" animals refers to those rats which showed no response to the injection of alloxan, despite the fact that they were given the same dose as the other animals. In the severely comatose animals, there was noted a marked elevation of blood sugar and of plasma inorganic phosphate, whereas in the liver there was a decrease in total acid-soluble phosphate, in the 7-minute hydrolyzable and residual phosphates, and in glycogen, together with a rise in liver inorganic phosphate. The 7-minute fraction appeared to consist chiefly of the labile phosphates of adenosine pyrophosphate (adenosine diphosphate and adenosine triphosphate). The decrease in this fraction may be attributed to a diminution of the oxidative reactions resulting from lack of insulin.[9] It will be observed that neither the presence of alloxan nor of dietary restriction per se accounts for the changes in the comatose animals.

TABLE 1*

	Number of rats	Blood sugar mgm per cent.	Plasma inorganic P mgm per cent.	Liver				
				Glycogen per cent.	Total acid soluble P†	Inorganic P	7-minute hydrolyzable P‡	Residual P§
Control animals	8	115 ± 2.4	8.7 ± 0.5	0.155 ±0.018	95.9 ± 2.2	24.2 ± 0.8	18.0 ± 0.6	54.1 ± 1.9
Alloxan refractive animals	5	105 ± 15.5	8.1 ± 0.7	0.128 ± 0.021	95.1 ± 1.4	23.3 ± 0.7	20.6 ± 1.1	51.6 ± 1.9
Comatose animals	6	770 ± 95.5	26.3 ± 2.4	.064 ± .013	73.4 ± 0.6	33.2 ± 1.3	6.2 ± 0.4	34.4 ± 2.1

* The measure of variability is the standard error of the mean.
† All liver P values are in mgm P per 100 grams of fresh liver.
‡ Consists chiefly of the labile P of adenosine pyrophosphate (mixture of adenosine triphosphate and adenosine diphosphate).
§ Residual P represents the difference in P after the 7-minute hydrolyzable and inorganic P have been subtracted from the total acid soluble P.

Table 2 gives the results when varying doses of insulin[10] are administered to the comatose rats. It is evident that such administration is followed by a significant fall in blood sugar and plasma inorganic phosphate. In addition there is a slight fall in the liver inorganic phosphate and an increase in the total

TABLE 2

EFFECT OF INSULIN THERAPY ON RATS IN DIABETIC COMA

Units* of regular insulin given intraperitoneally	Blood sugar mgm per cent.	Plasma inorganic P mgm	Liver				
			Glycogen per cent.	Total acid soluble P	Inorganic P	7-minute hydrolyzable P	Residual P
10	370	21.9	0.219	80.0	28.2	12.4	39.6
10–10	...	23.6	0.895	87.6	28.0	15.7	43.9
20–20	105	7.1	0.920	87.9	23.6	17.0	47.3
15–15–15	685	19.6	0.341	74.9	29.9	10.0	35.0
15–15–15	297	15.4	0.770	75.0	24.5	11.7	38.8
15–15–15	90	12.0	0.894	86.5	23.1	14.9	48.5
15–15–15	32	10.8	0.340	96.6	28.2	17.6	50.8
15–20–20	64	3.2	0.820	83.3	28.2	14.4	41.9
20–20–20	790	20.5	0.535	75.5	32.2	11.8	41.5
20–20–20	87.5	9.8	0.955	86.3	26.1	16.1	44.1
20–20–20	185	18.6	0.930	80.5	29.0	10.0	41.5

* Insulin was given every 1½ hours. The animals were sacrificed 1½ hours after the final dose.

[6] N. Nelson, S. Rapoport, G. M. Guest and I. A. Mirsky, *Jour. Biol. Chem.*, 291, 1942.
[7] N. O. Kaplan and D. M. Greenberg, *Am. Jour. Physiol.*, 140: 598, 1944.
[8] N. O. Kaplan and D. M. Greenberg, *Jour. Biol. Chem.*, 156: 525, 1944.
[9] N. O. Kaplan and D. M. Greenberg, *Jour. Biol. Chem.*, 156: 553, 1944.
[10] Insulin was generously supplied by Dr. W. A. Feirer, of Sharp and Dohme, Inc.

acid-soluble phosphate as well as the 7-minute hydrolyzable and residual phosphates. A marked rise in liver glycogen and considerable improvement of the clinical status of the animals was also noted. It is important to emphasize that enormous doses, up to 60 units of insulin, were necessary to bring about these improvements in comatose rats. It will be seen that although the degree of the response was not uniform the trends are definite.

SUMMARY

Alloxan given parenterally produces in the rat a state of diabetic coma which is analogous to the severe coma of human diabetes. Such animals, moreover, show a rise in plasma inorganic phosphate and blood sugar. They also show in the liver a decrease in glycogen and in the total acid-soluble phosphates. An increase in liver inorganic phosphate occurs with a concurrent fall in adenosine pyrophosphate and other organo-phosphates. Hence the rise in plasma inorganic phosphate during coma is at least partially due to a loss of liver phosphate. It is probable that this rise in plasma inorganic phosphate results from a breakdown of organo-phosphates which result from the depressed oxidations associated with insulin lack. Insulin, when administered in exceptionally large doses, tends to cause improvement in both the clinical and chemical state of comatose rats.

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AN EPIZOOTIC OF PNEUMOCOCCUS TYPE 19 INFECTIONS IN GUINEA PIGS[1]

OUTBREAKS of pneumococcal infections among guinea pigs have been known for some time to occur in this country and in Europe.[2] The epidemic strains and the pneumococci found in normal guinea pigs have not usually been classified serologically and those that were fell into the so-called Group IV. In 1932, Neufeld and Etinger-Tulczynska[3] identified the type 19 pneumococcus as the prevalent type in both healthy and infected guinea pigs of various laboratories and dealers in Berlin and elsewhere in Germany. At that time Webster could not find this organism in the nasal washings of guinea pigs in New York.[3] To our knowledge carriers of type 19 pneumococci or spontaneous infections with this organism have not been described among guinea pigs in the United States.[4]

The infections reported in this paper occurred among apparently healthy guinea pigs in the course of studies of certain effects of sodium salicylate in these animals. The occurrence of unexpected deaths among experimental and control animals prompted a bacteriological study which revealed that the type 19 pneumococcus was the cause of most of the fatal lesions. In addition, carriers of the same organism were found among apparently healthy guinea pigs. Sulfadiazine was used prophylactically in one group of animals in an attempt to eliminate the infections and to clear the carrier state.

Materials and Methods. The 3 groups of guinea pigs used in this study were procured from different dealers in widely separated localities. They were apparently healthy when received and no infections had previously been recognized in the stock from which they came. Nasal washings were obtained for culture in the first group as soon as the epizootic was recognized. In the other 2 groups they were done when the animals were first observed. These cultures were repeated at autopsy and, in addition, cultures were made from swabbings of the cut surfaces of the lungs, of the internal ears, nasal accessory sinuses and other purulent foci at that time. The nasal washings were done as described by Neufeld,[2] saline being used for the washings in the first group and broth in the other groups. The cultural methods for isolation and identification of the pneumococci were those described elsewhere.[1] Autopsies were done as soon as possible after the death of the animals or immediately after they were sacrificed (by stunning). Flamed instruments were used to cut the lungs and to open the bony structures before taking the cultures. The organs were examined grossly and also microscopically from Zenker's-fixed, paraffin sections stained with hematoxylin-eosin. The sodium salicylate and the sulfadiazine were fed to the animals by stomach tube, a separate tube being used for each animal. Their diets were not otherwise controlled. Cultures of the rabbit chow used to feed the animals yielded no pneumococci. The observations in each of the 3 groups will be presented separately.

Group I. The 20 guinea pigs in this group were delivered to the animal quarters at the Harvard Medical School on February 5, 1945, from a breeder in Saugus, Mass., which is about 10 miles north of Boston. After a few days they were brought to the animal house of the Neurological Unit at the Boston City Hospital, where they were kept thereafter in a

[1] From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Mass.

[2] For a review of the epidemiology of pneumococcal infections in animals see M. Finland, *Medicine*, 21: 307, 1942.

[3] F. Neufeld and R. Etinger-Tulczynska, *Ztschr. f. Hyg. u. Infektionkr.*, 114: 324, 1932.

[4] In 1938, Geoffrey Rake (*Am. J. Hyg.*, 28: 377) described spontaneous infections due to *S. enteritidis*, type 19 pneumococcus and *A. bronchosepticus* in a guinea pig breeding colony at the farm division of the Connaught Laboratories in Toronto.