doses of insulin (Verner and Engel, unpublished).

*Summary.* Epinephrine and norepinephrine stimulate production of nonesterified fatty acids from rat adipose tissue when incubated *in vitro* in rat plasma, presumably by stimulating the hydrolysis of neutral fats within the tissue. Incubation under nitrogen markedly inhibits this effect of epinephrine and appears to increase production of fatty acids from control tissues. The d-isomers of epinephrine and norepinephrine are much less active in this system than the physiological l-isomers. indicating a specificity in tissue response. Some implications of this lipolytic activity are discussed.

We are indebted to Drs. McC. Goodall and N. Kirschner for the hormones used.

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Received July 22, 1958. P.S.E.B.M., 1958, v99.

## On Nonantigenicity of Polysaccharides in Guinea Pigs*† (24356)

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Although antigenicity of polysaccharides has been investigated extensively in rabbits(1) and man (2a, b), there is little in the literature concerning antigenicity of polysaccharides in guinea pigs(3). The availability of good technics for immunization, production of "delayed hypersensitivity," and the detection of antibody prompted a reinvestigation of the problem from several approaches.

*Methods. Antigen and antibody.* Both pneumococcal polysaccharides SIII and rabbit anti SIII were obtained from Dr. M. Heidelberger. Dextran preparations B512F and 3079 were obtained from Northern Regional Research Laboratory and the Pharmacia Co. respectively. Human antisera against these materials were obtained by immunization of normal, healthy medical students(2b). Five times recrystallized ovalbumin (Ea) was prepared by the method of Kekwick and Cannan (4). Anti Ea sera were obtained from rabbits after a course of intravenous injections of alum precipitated Ea. The antibody content of various sera was determined by employing quantitative precipitin technics developed by Heidelberger and co-workers(5).

*Methods.* 1. Antigens used for immunization of guinea pigs were prepared several ways: a) Dextran preparation or pneumococcal SIII was mixed in a complete adjuvant mixture containing Arlacel C, Bayol F and dead mycobacteria(6,7). The final concentration of polysaccharide was 20 $\mu$g/ml. b) Specific precipitates of either dextran-anti dextran, SIII-anti SIII or Ea-anti Ea were prepared according to procedure employed by Uhr, Salvin and Pappenheimer(8). In this procedure precipitates are formed in antibody

* These studies were supported by research grants from N.I.H., U.S.P.H.S.

† Publication No. 176 of Department of Pathology, University of Pittsburgh School of Medicine.

TABLE I. Methods of Immunization Employed.

| Group* | Material inj.† |
|---|---|
| 1a<br>1b | 10 μg SIII with t.b. |
| 2a<br>2b | 10 μg SIII–anti SIII ppt. with t.b. |
| 3 | 10 μg SIII–anti SIII<br>10 μg Ea–anti Ea } with t.b. |
| 4 | 10 μg SIII–anti SIII<br>10 μg Ea–anti Ea } no t.b. |
| 5 | Dextran B512F–anti dextran ppt. |
| 6 | Dextran 3079–anti dextran ppt. |

* 6 guinea pigs used for each group.
† All inj. were in the foot pad.

TABLE II. Procedures Employed with Various Groups of Guinea Pigs.

| Group | Procedure employed |
|---|---|
| 1a, 2a | Guinea pigs bled D0, D10, D20, D30, D60. |
| 1b, 2b | Guinea pigs bled D0, D10 and D20. On D20, 10 μg SIII inj. into foot pads. Guinea pigs bled again on D30 and re-inj. with 10 μg SIII and bled again on D60. |
| 3, 4 | Guinea pigs bled D0. On D12 skin tested with 10 μg SIII and 10 μg Ea and bled again D13. On D19 guinea pigs were bled and skin tested with SIII, Ea and Rgg. |
| 5, 6 | On D13 guinea pigs bled and skin tested with dextran. On D15 guinea pigs challenged by intrav. inj. of 1 ml of human serum. |

excess where only 1/3 of antibody is precipitated with all of the added antigen. Specific precipitates which formed were washed in the cold 4-5 times, resuspended in saline and incorporated in an adjuvant mixture. In some instances, dead mycobacteria were not incorporated into the mixtures. 2. *Immunization of guinea pigs.* Male white guinea pigs weighing between 350-450 g were injected in the foot pads with adjuvant mixtures. Depending upon the group, each guinea pig received 10 μg of the polysaccharide, Ea, or a mixture of the two. Table I summarizes the various groups and injections administered. As noted, some guinea pigs were injected with free polysaccharide whereas others received the antigen-antibody precipitate. 3. Table II summarizes the various treatments that each group of guinea pigs received. 4. *Analysis of serum.* Table III summarizes the results of testing the sera. Both *in vitro* and *in vivo* methods were employed to determine whether or not the giunea pigs had produced detectable amounts of antibody. The *in vitro* precipitin technics for detecting antibody were similar to those developed by Heidelberger and MacPherson for analysis of human sera (9). In addition, micro modification of the technic of double diffusion in agar as described by Preer was employed(10). As the above

TABLE III. Results Obtained by Different Methods of Analysis of Guinea Pig Serum.

| | | Methods employed in analyzing the sera | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Micropptn. reaction | | | Agar diffusion | | | P. C. A. | | |
| Group | Day of bleeding | SIII or dextran | Ea | Rgg | SIII or dextran | Ea | Rgg | SIII or dextran | Ea | Rgg |
| 1a, 1b | 0 | — | | | — | | | — | | |
| | 10 | — | | | — | | | — | | |
| | 20 | — | | | — | | | — | | |
| | 30 | — | | | — | | | — | | |
| | 60 | — | | | — | | | — | | |
| 2a, 2b | 0 | — | | | — | | — | — | | — |
| | 10 | — | | | — | | + | — | | + |
| | 20 | — | | | — | | + | — | | + |
| | 30 | — | | | — | | + | — | | + |
| | 60 | — | | | — | | — | — | | + |
| 3 | 0 | — | — | — | — | — | — | — | — | — |
| | 12 | — | — | + | — | — | + | — | — | + |
| | 19 | — | + | + | — | + | + | — | + | + |
| 4 | 0 | — | — | — | — | — | — | — | — | — |
| | 12 | — | — | ± | — | — | ± | — | — | + |
| | 19 | — | + | + | — | + | + | — | + | + |
| 5 & 6 | 13 | — | + | — | | | + | — | | + |
| | 15 | Guinea pigs showed systemic anaphylaxis to human serum. | | | | | | | | |

TABLE IV. Skin Reactions Observed in Guinea Pigs.*

| Group | Guinea pig | Time of reaction, day 12 | | | | Time of reaction, day 19 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 hr Rx | | 24 hr Rx | | 6 hr Rx | | | 24 hr Rx | | |
| | | Ea | SIII | Ea | SIII | Ea | SIII | Rgg | Ea | SIII | Rgg |
| 3 | 1 | 3x3 | 4x4 | 18x18 | 7x7 | 15x15 | 10x10 | 12x12 | 12x12 | 8x8 | 15x15 |
| | 2 | 12x12 | 5x5 | 25x25 | " | 18x18 | " | " | 10x10 | 10x10 | 12x12 |
| | 3 | 10x10 | 4x4 | 22x22 | 8x8 | | | | | | 15x15 |
| | 4 | 3x3 | 3x3 | 20x20 | 7x7 | 12x12 | " | 15x15† | 12x12 | 8x8 | 5x5 † |
| | 5 | 10x10 | 5x5 | " | 10x10 | 17x17 | 12x12 | 14x14 | — | 4x4 | 15x15 |
| | 6 | " | 4x4 | " | 7x7 | | | | | | |
| 4 | 7 | — | — | — | 6x6 | | | | | | |
| | 8 | — | — | 4x4 | " | " | " | 15x15 | | | |
| | 9 | — | — | 3x3 | 7x7 | 8x8 | 8x8 | 7x7 | | | |
| | 10 | 3x3 | 7x7 | 4x4 | 8x8 | 12x12 | 12x12 | 10x10 | | | |
| | 11 | " | 6x6 | 3x3 | " | 8x8 | " | 12x12 | | | |
| | 12 | 4x4 | 7x7 | 4x4 | 7x7 | | | | | | |

* All reactions expressed as size of erythema in mm. † Necrosis.

procedures can only detect precipitating antibody, the *in vivo* technics of passive cutaneous anaphylaxis (PCA) and systemic anaphylaxis were also employed. These latter technics are known to detect exceedingly small amounts of non precipitating antibody. 5. *Skin testing of guinea pigs.* At appropriate times after injection as outlined in Table IV, guinea pigs were injected intradermally with 0.1 ml of solutions containing 10 $\mu$g of appropriate antigen, *i.e.,* polysaccharide, Ea or rabbit gamma globulin (RGG). Skin reactions were read at 5-6 hours and again after 24 hours. The various results will be discussed below.

*Results.* The data presented in the present report confirm the published information on the poor antigenicity of purified polysaccharides in guinea pigs. The methods employed previously did not use as vigorous technics for immunization nor as rigorous methods for analysis of the serum as employed in the present report. In the present study, none of the combinations of methods of immunization used produced detectable amounts of antibody. The most sensitive technic for detecting antibody appears to be the passive cutaneous anaphylaxis test as developed by Ovary(11). In our hands, too, the method could detect 0.003 $\mu$g of anti Ea and 0.02 $\mu$g of anti SIII.

Whereas purified preparations are not antigenic in rabbits(1), only small amounts of polysaccharide material are necessary to produce considerable levels of circulating antibody in humans(2). The reason for this difference in behavior is not known, but it should be realized that there are definite differences among species in the response to purified antigens whether they be protein(12), polysaccharide or synthetic materials(13). It should also be mentioned that if polysaccharides are injected either as part of the bacterial cell, or adsorbed onto collodion then antibody against the polysaccharide is produced(14). However, the particulate nature of the antigen is not the only factor determining whether a polysaccharide is to be antigenic in guinea pigs. Although guinea pigs were injected with specific precipitates they responded only to rabbit gamma globulin. This latter observation is important as it shows that the animals were not unreactive.

Although the present significance of the phenomenon of "delayed hypersensitivity" produced by injection into guinea pigs of appropriately prepared antigen-antibody precipitates is still somewhat controversial, the data in Table IV do show that the phenomenon can be reproduced. Definite "delayed reactions" to Ea were observed in guinea pigs injected with Ea-anti Ea precipitates plus dead mycobacteria, whereas a poor or negligible reaction was observed when mycobacteria were omitted from the adjuvant preparation. A more detailed report of this phenomenon will be presented separately. The data do show, however, that no significant "delayed reactions" were observed against

SIII. The slight increase in skin reactions with the guinea pigs on D19 is at present unexplainable.

*Summary.* The lack of antigenicity of purified pneumococcal polysaccharide and dextran in guinea pigs has been demonstrated. Both the methods used for immunization and detection of antibody have been shown to be quite good for the other systems.

The authors wish to thank Mr. Albert Nordin for his competent technical assistance and Dr. M. Heidelberger for gifts of SIII and rabbit anti SIII serum.

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Received August 4, 1958. P.S.E.B.M., 1958, v99.

## Purification of a Rheumatoid Factor.* (24357)

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The serological reactions of the rheumatoid factor have been studied extensively not only for the promise they hold as a diagnostic tool, but also for their possible bearing on the etiology of rheumatoid arthritis. A critical review of past work has appeared recently(1). The rheumatoid factor is a macroglobulin with a sedimentation coefficient of $s_{w,20} =$ 19 S; in many instances it circulates in the blood as a complex ($s_{w,20} =$ 22 S) containing other gamma globulin(11). The rheumatoid factor, furthermore, contains 2 serologically distinct and separate components(2,3,4). While both components react with aggregated gamma globulin(5,6), only one seems involved in adsorption onto certain antigen-antibody systems(3). Such systems form the basis of the various sensitized erythrocyte(7,8) and bacterial agglutination reactions(9). A number of investigations aimed at isolation of the rheumatoid factors have been reported recently(4,10,12,13,14). A new simple method is reported herewith, which avoids the compli-

* Supported in part by grant from N. I. of Arthritis and Metabolic Diseases, U. S. P. H. S., Bethesda, Md.