A GAS THERMOSTAT

A SIMPLE yet efficient device for accurate thermal control of ovens and liquid baths consists simply of the bulb of a flask sealed onto a 10 mm tube which is bent to form a complete loop, and then rises to a gas outlet through a side tube and a gas inlet in the end of the tube. In the loop of the tube enough mercury is trapped to fill each limb slightly less than half full. The gas enters the end of the tube through a smaller tube, which extends down almost to the surface of the mercury. When the bulb is heated, the air in it expands and pushes the mercury against the end of the inlet tube, shutting off the gas flow which normally flows down the inner tube, back between the inner and outer tubes, and out through the side tube to the burner. A by-pass consisting of two glass T's and a screw clamp serves to keep the burner lighted when the mercury shuts off the main flow of gas.

The thermostat operates by simply heating to 10–15° C. higher than the desired temperature. During this heating the mercury is pushed into one limb of the loop, after which the confined air escapes past the mercury. When the temperature is lowered to the operating temperature, the mercury is equalized in the two limbs. The inner gas conduction tube is then lowered until it almost touches the surface of the mercury. Any subsequent change in temperature will vary the flow of gas, causing a compensating heating effect.

This device is ideal for variable operating temperatures, as no adjustments are required to effect the change. With a 250 cc bulb it will maintain the temperature within less than 0.1° C. of the desired temperature.

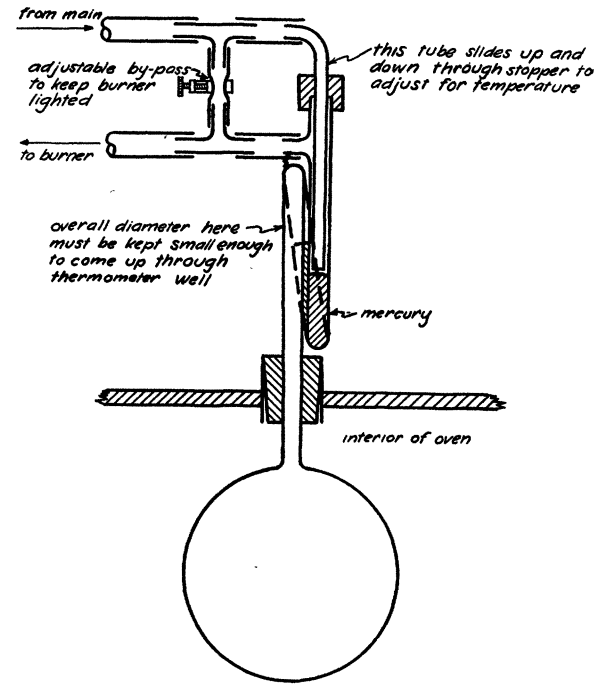

FIG. 1.

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SPECIAL ARTICLES

PNEUMOCOCCUS ANTIBODIES—WHAT ARE THEY?[1]

STUDIES on serum antibodies and antitoxins have followed two lines of endeavor. The first is the isolation and purification by means of well-known protein precipitants, and the second is the dissociation of the antibody-antigen complex. The former has led to concentration of immune sera for clinical purposes, but, so far, has given but little information in regard to the chemical nature of antibodies. On the other hand, study of the dissociation of the antigen-antibody complex has given fruitful results.

Significant work on pneumococcus antibody began with the observations of Gay and Chickering, and of Chickering, in their attempts to dissociate the protective substance from the pneumococcus antibody complex obtained by the usual method of agglutination. They found that by treating the pneumococcus cell-antibody precipitate with sodium carbonate at 42° C., a clear solution was obtained which contained most of the protective antibodies of antipneumococcus serum. These authors showed that this solution contained both protein and antigen. Huntoon and his associates continued this study on a larger scale. But, unlike Gay and Chickering, they claimed that their antibody solution, containing the protective substance of the immune serum, was devoid of agglutinins, was free from protein and, in addition, was resistant to the action of pepsin and trypsin. In interpreting Huntoon's negative results it is well to bear in mind that "test for protein, negative" does not necessarily mean "protein absent," because the usual tests for protein are not highly sensitive.

In our studies the antigen-antibody complex of pneumococcus has been viewed as a definite chemical compound, a resultant new chemical made up of the two reacting components. As shown by numerous investigators, this antigen-antibody compound is but

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slightly soluble in physiological salt solution. Inasmuch as all serum proteins are completely soluble in this salt solution, the antibody complex may be washed free from inactive proteins present in the serum. Yet when the precipitate, formed by the mixture of antipneumococcus horse serum and antigen, is washed with physiological salt solution until the supernatant fluid has a constant minimum amount of nitrogen, this final precipitate still contains both protein and precipitating antigen. The antigens used were: washed pneumococcus cells, the polysaccharide of Heidelberger and Avery and the C substance of Tillett. The results of such an experiment are given in Table I. Here it is shown that the washed precipitate contained protein, measured in terms of nitrogen, from 7.6 per cent. to 1.4 per cent. of the total serum nitrogen in the case of Type I, and from 7.4 per cent. to 0.7 per cent. in the case of Type II. Yet the nitrogen content of the antigens used was negligible, except in the test with washed organisms. In this case, it was assumed that all the pneumococci used were precipitated by the antibody, and from the total nitrogen of the precipitate was subtracted the nitrogen content of the microorganisms. Since one serum was used in all the tests and since conditions were such that in each case maximum precipitation was obtained, the possibility is indicated, from the variation of the amount of protein precipitated by these antigens, that there may be present more than one antibody in the serum of a horse immunized against the entire pneumococcus cell. It appears that there is an antibody for each antigenic substance found in the microorganism.

TABLE I

AMOUNT OF NITROGEN IN PROTEIN PRECIPITATED BY VARIOUS ANTIGENS, TYPES I AND II

Antigen	Type I		Type II	
	Nitrogen in washed precipitate	Per cent. of total serum nitrogen*	Nitrogen in washed precipitate	Per cent. of total serum nitrogen*
	(mgs./cc)		(mgs./cc)	
Polysaccharide from broth	0.58	4.6	0.89	7.4
Polysaccharide from organisms	0.92	7.6	0.75	6.2
C substance (Tillett)	0.17	1.4	0.09	0.7
Whole cell	0.75	6.2	0.84	7.0

* Serum used contained 12 mgs of nitrogen per cc. Nitrogen determinations were on washed precipitate made up to one fifth the volume of serum used and corrected to equal nitrogen in the precipitate formed from 1 cc of serum with the various antigens.

If one considers the antibody-antigen complex as a chemical compound, then the possibility exists that the two reacting chemicals of this compound can be separated. When the precipitinogen is the polysaccharide of Heidelberger and Avery, the reacting substances are polysaccharide and immune protein. It has been found that alkali dissociates this complex and that calcium phosphate in an alkaline solution precipitates most of the polysaccharide, leaving in solution part of the protein which is now readily soluble in a solution of sodium chloride. The results of two experiments, which deal only with the amount of salt soluble protein separated, are given in Table II. In the first experiment (A), it is seen that with Type I 85 per cent. of the total protein present in the complex was separated from the precipitating antigen and 84 per cent. with Type II.

TABLE II

DISSOCIATION OF ANTIGEN-ANTIBODY COMPLEX

	Type I	Type II
Experiment A		
Nitrogen in antigen-antibody complex (mgs./cc)	3.09	3.2
Nitrogen in salt-soluble dissociated protein (mgs./cc)	2.62	2.75
Per cent. of total nitrogen in antigen-antibody complex	85.0	84.0
Experiment B		
Nitrogen in washed antigen-antibody complex (mgs./cc)	2.85	2.65
Nitrogen in salt-soluble dissociated protein (mgs./cc)	1.12	0.87
Nitrogen in protein precipitated a second time with SSS (mgs./cc)	0.96	0.60
Per cent. of nitrogen in the washed precipitate of dissociated protein	84.9	70.4

That the antigen-antibody reaction is reversible in nature is shown in the second experiment (B). For with both Type I and Type II, the dissociated salt soluble protein was reprecipitated with the addition of some of the original antigen, the polysaccharide of Heidelberger and Avery. The following cycle of events compels the conclusion that at least one antibody of antipneumococcus horse serum (anti-SSS) is protein in nature: first, the formation of a precipitate by mixing two soluble compounds, immune serum and antigen; second, the washing of this salt insoluble precipitate to remove the inert protein; third, the separation of the washed precipitate into two soluble

compounds, a salt soluble protein and the water soluble precipitating antigen; and fourth, the recombining of these two dissociated compounds to form again a precipitate which is but slightly soluble in physiological salt solution. So far there is no evidence to contradict the conception that this so-called anti-SSS antibody is protein.

The protein so isolated from the antigen-antibody complex was found to have an isoelectric zone between pH 6.8 and 7.4, and to be but slightly soluble in water, readily soluble in neutral salt, completely precipitable with 44 per cent. of saturation with ammonium sulfate and soluble in saturated sodium chloride. In addition, both pepsin and trypsin digest the protein with a resulting loss of immunological characteristics. Although not all immunological tests have been pursued with this dissociated protein, positive reactions were obtained indicating the presence of agglutinins, precipitins, bacteriolysins, opsonins, complement-fixing bodies and protective antibody. Accordingly, our results indicate, in the case of pneumococcus antibody, a confirmation of the unitarian theory, sponsored by Zinsser, that one antibody reacting with a single antigen is responsible for the usual immunological reactions. Whether or not any of the often suggested possibilities with regard to the character of the immunological material called antibody are eventually found to be true, according to our observations the answer to the question raised in the title is that the antibodies found in antipneumococcus horse serum are protein in nature.

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STROPHANTHIN. XXIX. THE DEHYDROGENATION OF STROPHANTHIDIN

EARLIER studies of the dehydrogenation of strophanthidin with selenium according to the method of Diels, Gädke and Körding[1] have been reported from this laboratory.[2] In this work a hydrocarbon was isolated which, although bearing strong resemblance to the $C_{18}H_{16}$ hydrocarbon (methylcylopentenophenanthrene) of Diels, Gädke and Körding obtained from cholesteryl chloride, differed from it in a number of respects. Its picrate melted at 138–140° instead of 118°, and it yielded on oxidation with chromic acid a red quinone, which in turn yielded a quinoxaline. The conclusion was reached, therefore, that this substance is probably a dimethylphenanthrene. However, in view of certain observations which will be presented in another connection, we were on the point of extending our study of the dehydrogenation of strophanthidin and its derivatives when the recent work of Tschesche[3] appeared. The latter, among other things, dealt with the dehydrogenation of dianhydrouzarigenin. Tschesche and Knick isolated from the reaction mixture a hydrocarbon which was shown to be identical with the $C_{18}H_{16}$ hydrocarbon obtained from the sterols and the bile acids. On the basis of the similarity in properties of the saturated desoxylactone obtained from one of the hydrogenation products of dianhydrouzarigenin (the so-called α_2-lactone) with octahydrotrianhydroperiplogenin,[4] which we had sent to Tschesche at his request for comparison, he concluded that a direct relationship was therefore established between uzarigenin and the other cardiac aglucones. And since the hydrocarbon $C_{18}H_{16}$ is now regarded as a characteristic dehydrogenation product of the sterol ring system, the conclusion was drawn that the cardiac aglucones are built on the same ring system as the sterols, a possibility which has, of course, been under constant consideration by ourselves and others. However, since discrepancies remained in the rotations and melting points which he reported between his α_2-lactone from uzarigenin and our octahydrotrianhydroperiplogenin (the melting point of which, 176–177°, had remained unchanged after three additional recrystallizations), we believed that confirmation was necessary.

This accordingly precipitated our reinvestigation of the dehydrogenation of strophanthidin with selenium. In our earlier work a procedure had been used which yielded in our preliminary trials the hydrocarbon $C_{18}H_{16}$ from cholesteryl chloride without difficulty. However, in our recent work we departed from this procedure by a strict observation of temperature conditions. The exact details will be described more fully in another place. One hundred fifty gms of strophanthidin gave 72 gms of oil, which distilled up to 275° at 1 mm. Repeated fractionation gave a fraction boiling at 185–195° at 0.2 mm. Crystalline material was obtained from this oil which after purification through the picrate was submitted to extensive fractional crystallization from 95 per cent. alcohol, according to the triangle scheme. One half gm of material melting at 124–125° (cor.) was finally obtained. This substance did not yield a red quinone on oxidation and by conversion into the picrate and trinitrobenzol and trinitrotoluol addition products was shown to be identical with the hydrocarbon $C_{18}H_{16}$ of Diels, Gädke and Körding.

Analysis:		
$C_{18}H_{16}$.	Calculated.	C 93.10, H 6.89.
	Found.	" 93.12, " 7.05.
		" 92.98, " 7.11.

[1] O. Diels, W. Gädke and P. Körding, *Ann. d. Chem.*, 459: 1, 1927.

[2] W. A. Jacobs and E. E. Fleck, SCIENCE, 73: 133, 1931; *Jour. Biol. Chem.*, 97: 57, 1932.

[3] R. Tschesche, *Z. physiol. Chem.*, 222: 50, 1933; R. Tschesche and H. Knick, *Z. physiol. Chem.*, 222: 58, 1933.

[4] W. A. Jacobs and N. M. Bigelow, *Jour. Biol. Chem.*, 101: 700, 1933.