cc of 15% NaOH is added. Both samples are then shaken immediately with 13 cc butyl alcohol for 3 minutes. The mixture is then centrifuged to separate the butyl alcohol layer and this is treated with anhydrous Na_2SO_4 to remove traces of water, and is allowed to stand in the dark for 20 minutes. Fluorescence is then determined in a Pfaltz and Bauer fluophotometer, the source of light for which is a mercury vapor bulb (General Electric, type H3-85 watts) shielded by a Wood filter (Jena UG-2), the emitted fluorescence being measured after the interposition of a double filter of bright bluish green (Jena BG-14) and bright yellow (Jena GG-3). The difference in fluorescence between the sample treated with alkali and that not so treated represents the fluorescence of the unknown compound. Comparative quantitative measurements can be obtained by comparing the fluorescence with that of a quinine sulfate solution containing 10 to 25 μg % in 0.1 normal H_2SO_4. The daily output of the unknown substance in urine of normal adults gives a fluorescence corresponding roughly to 100 μg of quinine sulfate. After the ingestion of 50 mg of nicotinic acid, the concentration in urine reaches 8 to 10 times its previous value, during the first 4 hours.

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A Simple Method of Preparing Dried Serum Proteins for Therapeutic Use.

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Attempts to preserve serum proteins in a dry form for therapeutic use have been made for a number of years. Flosdorf and Mudd's[1] method of preparing "lyophile" serum is for the most part satisfactory, but requires complicated and expensive special apparatus which was not at our disposal when we began to work with dried serum proteins. We therefore found it necessary to find or devise some method which could be carried out with ordinary laboratory apparatus. After preliminary trials with several other methods, we found the method devised by Hartley[2] for the preparation of dry and lipid-free immune sera to be most suitable,

[1] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[2] Hartley, P., *Brit. J. Exp. Path.*, 1925, **6**, 181.

and we have employed it, with slight modifications of the original method, since 1937.

A mixture consisting of 7 volumes of 95% alcohol and 3 volumes of redistilled absolute ether is cooled to –20°C or –18°C. To this mixture 1 volume of serum, previously cooled to 4°C, is added drop by drop and very slowly, the contents of the container being vigorously stirred for the entire time. After all the serum has been added, the flask is well shaken and allowed to stand for 2 hours.

The resulting fine white precipitate of serum proteins is filtered off on a Büchner funnel and is washed repeatedly with absolute ether cooled to –20°C. Since as much alcohol as possible should be removed, it is desirable, whenever practical, to use 20 volumes of absolute ether for the washings at this stage. The filter paper with the protein is placed over sulphuric acid in a vacuum desiccator, which is then evacuated to remove the remaining ether. Solid carbon dioxide is used to cool the precipitating and washing fluids.

When this procedure was carried out with small amounts of serum (10-20 cc), the protein preparation obtained was in the form of a coarse white powder, which was slowly soluble. A solution of this preparation was perfectly clear and resembled the original serum in appearance. Clear solutions can still be obtained from the dried preparations made 2 years ago.

Three experiments were carried out to test the physiologic properties of dried proteins prepared by this method from dog serum. (1.) Ten gram portions of dried serum were dissolved in physiologic saline solution by prolonged stirring, after which the solution was centrifugalized for 30 minutes at about 3,000 rpm to remove any undissolved protein present. It was then filtered through a Seitz bacteriological filter and the protein content was checked by the colorimetric method of Johnston and Gibson.[3] Twenty cc of the solution (2 g of protein) were injected intravenously into a dog after a preliminary temperature observation had been recorded. No alterations in temperature were observed in observations made at 15-minute intervals for several hours after the injection. (2.) The experiment was repeated with a different protein solution on another dog without ill effects. (3.) A third dog was given 50 cc of solution (5 g of protein) representing a combination of 2 other protein preparations. A transitory temperature elevation of 1°C was observed.

Experiments were then carried out with human serum proteins of appropriate blood type, which were dried, dissolved, Seitz-filtered,

[3] Johnston, G. W., and Gibson, R. B., *Am. J. Clin. Path. Tech. Suppl.*, 1938, **8**, 22.

tested for sterility, and injected into a dog. When no reaction was observed in the animal, 40 cc of the same solution (4 g of protein) was injected intravenously into a patient. There was no apparent reaction.

When serum was used in 500-700 cc portions, some difficulty was encountered in the preparation of dried serum proteins due to denaturation of the proteins. The cause of the denaturation is probably the difficulty of stirring the larger amount of precipitating fluid adequately, as well as difficulties in filtration. If filtration is not accomplished very rapidly, the alcohol in the precipitating fluid tends to become warm enough to denature the serum proteins. These difficulties have not yet been entirely overcome.

Summary. The method devised by Hartley for the preparation of dry and lipid-free immune sera has been adapted to the preparation of dried serum proteins in quantity. In spite of some technical difficulties, the serum proteins prepared by this method have been injected into dogs and into a single human subject without serious reactions.

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Resistance of Human Spermatozoa *in vitro* to Sulfanilamide and Sulfapyridine.

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Jaubert and Motz[1] studied the effect of sulfanilamide on spermatogenesis in 23 men suffering from gonorrhea. They noted a reduction in both the number and vitality of the spermatozoa, with their complete immobilization in some instances. Marion, Barbellion, and Torres,[2] observed that small oral doses of sulfanilamide caused a decrease in the number and motility, and an increase in the abnormal forms, in the spermatozoa of 69% of their patients. Vigoni[3] found the same changes in men treated by urethral irrigation with sulfanilamide, 2 of his patients actually developing azoospermia.

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1 Jaubert, A., and Motz, C., *Presse méd.*, 1938, **46**, 237.

2 Marion, Barbellion and Torres, *Bull. Soc. fran. d'urol.*, May 16, 1938.

3 Vigoni, M., *J. Belge d'urol.*, 1938, **11**, 375.