

optimal sodium chloride retention to restore body chlorides to normal in patients with hypochloremia. On the basis of this formula it has been found that for clinical purposes the administration of 0.5 gm. of sodium chloride per kilo of body weight for each 100 mg. % that the plasma chlorides need to be raised may be expected to restore the body chlorides to normal.

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Further Observations on Intranuclear Inclusions Produced by Non-Virus Materials.

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The purpose of this report is to record certain additional findings obtained in the application of the technic previously reported¹ for producing intranuclear inclusion-bodies with substances unassociated with a virus. This procedure consisted essentially of the injection of autoclaved materials subcutaneously into guinea pigs and the subsequent removal of nodules so produced for histological examination. When aluminum hydroxide or alundum was used, intranuclear inclusion-bodies were always found in the mononuclear and giant phagocytic cells of the tissue-reaction about one week after injection and usually as long as the nodules persisted. With ferric hydroxide and carbon they were found but irregularly or in small numbers. Histochemical reactions and morphological characteristics of such inclusions resembled very closely those of viral diseases. However, transmission and other experiments disclosed no virus in association with these inclusions.

Following is a list of additional materials which have been similarly injected subcutaneously into guinea pigs in order to determine whether the resulting tissue-reactions would also contain inclusions: suspensions of normal brain tissue of mice, of guinea pigs, and of rabbits (fresh or autoclaved); normal rabbit tissues (liver, spleen, kidney, testicle); commercial lecithin; alcoholic extract (lipins) of monkey brain; phosphatide of tubercle bacilli;* and 1:2:5:6 dibenzanthracene.† Although the reactions to these substances differed

¹ Olitsky, P. K., and Harford, C. G., *Am. J. Path.*, 1937, **13**, 729.

* Kindly supplied by Dr. A. L. Joyner.

† Kindly supplied by Dr. A. Claude.

somewhat from one another and from that of aluminum hydroxide, they all contained mononuclear phagocytic cells and in certain instances also giant phagocytic cells. Of all the materials, only brain tissue gave reactions containing the characteristic intranuclear inclusions. These bodies were usually few in number and difficult to find, although some sections of the induced nodules contained moderate numbers while in others none were found. Inclusions occurred with rabbit and guinea pig brain, fresh or autoclaved, injected into guinea pigs but not with mouse brain. It was thought that the lipins of cerebral tissue might have caused the inclusions but the results do not show this; the constituent of this material which induces the bodies is still unknown.

It has been shown by Smithburn and Sabin² that the phosphatide of tubercle bacilli, when injected into the connective tissues of guinea pigs, is ingested by phagocytic cells which can be identified by the supravital staining technic as epithelioid cells. As previously noted,¹ the cells that ingest aluminum hydroxide in the subcutaneous tissues of guinea pigs are also of the epithelioid variety, as determined by the supravital technic. The fact that the epithelioid cells in the phosphatide lesion do not show intranuclear inclusions, while those in the lesion of aluminum hydroxide do show them, indicates that these bodies in the nucleus are not merely characteristic of epithelioid cells in the subcutaneous tissue of the guinea pig but are associated with the presence of certain foreign substances.

That phagocytic cells in sites other than subcutaneous tissue may contain intranuclear inclusions after engulfing aluminum hydroxide is indicated by the following: Aluminum hydroxide has been injected intratesticularly into the peritesticular tissues, and into the pericardium of guinea pigs. Localized nodular masses were formed consisting of mononuclear and multinuclear phagocytic cells actively engulfing the foreign substance. The reactions resembled those in subcutaneous nodules, and the nuclei of the phagocytic cells contained similar inclusions. Although rabbits responded with the same type of tissue-reaction in the testicles as guinea pigs did, inclusions could not be found. Mice receiving aluminum hydroxide subcutaneously showed tissue-reactions similar to those of the guinea pig and the rabbit but in this animal intranuclear inclusion-bodies were seen only occasionally.

Dilutions of suspensions of aluminum hydroxide were injected subcutaneously into guinea pigs in order to make a rough determination of how small an amount would produce inclusions. The least

² Smithburn, K. C., and Sabin, F. R., *J. Exp. Med.*, 1932, **56**, 867.

amount to give a local reaction with inclusions was that equivalent to 0.01 mg. of Al_2O_3 . Alundum is also a form of Al_2O_3 and is commonly used as an abrasive in the preparation of tissues for animal inoculation. In the usual procedure, however, it is completely removed from the tissue-suspensions by centrifugation.

Summary. The following non-virus materials were used in guinea pigs in addition to those already reported: suspensions of normal brain tissue; normal rabbit tissues (liver, spleen, kidney, testicle); commercial lecithin; alcoholic extract (lipins) of monkey brain; phosphatide of tubercle bacilli; and 1:2:5:6 dibenzanthracene. Of these only brain induced characteristic intranuclear inclusions. Aluminum hydroxide injected into other sites than the subcutaneous tissues of guinea pigs produced similar reactions with inclusions. The mouse responded only feebly with inclusions on injection of aluminum hydroxide while the rabbit again failed.¹ The approximate minimal effective dose of Al_2O_3 needed for production of the intranuclear bodies was found to be 0.01 mg. per cc. The various aspects of these findings are discussed.

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Relief of Myxedema and Cretinism by Iodinated Blood Serum.*

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Since Baumann's finding that thyroglobulin contains iodine, many investigators¹ have sought for the chemical mechanism by which hormonal activity is produced when the native protein is iodinated in the body. The isolations of (inert) diiodotyrosine and (active) thyroxine by hydrolysis of thyroglobulin, together with the formation of artificial thyroid protein from diiodotyrosine peptone² have suggested that under certain conditions the phenolic groups of diiodotyrosine may undergo conjugation to form an iodized thyronine nucleus. The rapid formation of active thyroid protein which occurs in glands of patients with exophthalmic goiter when iodine is

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¹ Hutchison, R., *J. Physiol.*, 1898-99, **23**, 178.

² Salter, W. T., and Pearson, O. H., *J. Biol. Chem.*, 1936, **112**, 579.