killed in order to determine whether metastasis from the first inoculation had taken place. The results of this series of experiments show that secondary inoculation succeeds in a high percentage of the rats in which no visible metastases can be seen, and in which visible metastases, in the lungs chiefly, are present. The exact figures will be given in the complete publication to be issued soon.

The results of this series of experiments bear upon the view expressed by Sticker, that a primary tumor protects the body from the development of a secondary tumor until the period of metastasis arrives, and upon Ehrlich's negative results in secondary transplantations of a rapidly growing mouse carcinoma. The sarcoma of these experiments is characterized by its infiltrative growth, but it increases far less rapidly than the most active of Ehrlich's tumors, and reaches, in relation to the size of the rat, no such large size as the latter does in proportion to the size of the mouse.

34 (177)

On certain chemical complementary substances.

By **HIDEYO NOGUCHI.**

[*From the Rockefeller Institute for Medical Research.*]

In blood serum there is a constituent known as complement or alexin, which dissolves blood corpuscles or bacteria when the latter are properly sensitized. Its existence can only be demonstrated by the aid of immune bodies or amboceptors. The action of complement disappears when the serum gets old or is heated to 56° C. for a short time. The fate of complement after inactivation is not known. Complement is generally believed to undergo disintegration. Blood serum yields upon warm alcoholic extraction a substance or a group of substances of powerful lytic activity. The same is also true of leucocytes, glands and certain visceral organs. On account of some differences existing in the lytic mechanism and thermal resistance between genuine serum complement and alcoholic "extract lysins," no direct comparison has been made to establish a possible relationship between these two constituents. Complement is lytic only in the presence of immune

bodies, while the extract lysins are active by themselves. The action of complement diminishes with age and is destroyed by a temperature of about 56° C., whereas the extract lysins do not deteriorate with age or on boiling. So the general conception of to-day is that they are entirely distinct classes of bodies. Up to the present, no account of the parts which may be played by the other serum components has been taken into consideration. A comparison made under different conditions is devoid of value, and observations on this point seem desirable. I have therefore subjected both complement and the "extract lysins" to a comparative study under the same conditions. I have also identified the chemical nature of various "extract lysins," and pure chemical preparations have been subjected to a similar comparative study. My method of obtaining lytic substances from the blood or other organs was carried out as follows:

To one volume of blood or thick emulsion of any organ, three volumes of 95 per cent. alcohol are added. The mixture is left for about a week at 45 or 50° C. Then the filtrate is evaporated to dryness. The dried mass is extracted with hot alcohol. The alcoholic extract is dried. The dried mass is extracted with ether. The ether insoluble fraction is usually highly lytic, while the other fractions are inactive. The last, ether insoluble, hot alcohol soluble fraction is, of course, free from salts, proteins, neutral fats, fatty acids, cholesterin and its esters, lecithin and other phosphorized fats. It is soluble in water or 0.9 per cent. saline solution with slight opalescent appearance, and is neutral to litmus. Chemically, this fraction represents various soaps. The addition of acetate of lead and subsequent ethereal extraction removes its original lytic substance. Any strong acid produces a milky appearance due to the splitting of the soapy substance, and its hemolytic activity is reduced. Osmic acid gradually turns the solution dark. The solution yields a thick precipitate with phosphotungstic acid and with bromine. Millon's test is negative. This fraction, therefore, consists of various soluble soaps derived from the blood and organs.

My experiments with various soap fractions of the blood and organs show that such fractions possess considerable lytic activity when employed in 0.9 per cent. saline solution. The corpuscles

used were always washed free from the serum, as the latter paralyzes the lytic action of the soap fraction. It was found that the addition of an adequate quantity of indifferent or non-specific serum to the extract removed the lytic property of this fraction. But this inactivation was again found to be only superficial, for the extract was not inactive upon the corpuscles which had been treated with specific or normal amboceptors, nor was it inert in the presence of suitable immune bodies. In other words, this soap fraction acquires the property of acting as a complement. This artificial complement can easily be inactivated by heating it to 56° C. for half an hour, or by leaving it for a week or longer at room temperature. Its complementary action is absent at 0° C. Like serum complement, it becomes inactive when mixed with adequate quantities of various alkali earth salts of strong acids, and any acid stronger than carbonic acid. Alkalies delay the complementary action of this mixture. It may be stated here that the soap fraction in a protein-free solution cannot be inactivated by acids or alkalies. Without the serum proteins, no inactivation at 56° C. or on account of age or by suppression of its action at 0° C. can be obtained. All these characteristics of a complement are possibly to be ascribed to the serum proteins which are present.

My experiments with pure preparations of various soaps not only strengthen the above findings, but they further furnish explanation of the inactivation processes of various alkali earth salts upon complement and the soap fraction of the blood or organs. In this series of experiments, I have employed stearates of sodium, magnesium, calcium and barium, and oleates of ammonium, neurin, sodium, magnesium, calcium and barium. With the exception of certain alkali earth soaps, they are soluble with opalescence in 0.9 per cent. saline solution. Oleate soaps are, as a rule, more easily soluble than the corresponding stearates. As regards their hemolytic activity, it may be stated that the oleates are nearly as much as ten times more powerful than the stearates, and that all insoluble soaps are without lytic action. Of the oleates, neurin soap is the most soluble, and ammonium soap the least. These soluble oleate soaps were used in 1/100 to 1/200 N solutions. 0.5 c.c. of 0.1 per cent. solution (ca. 1/300 N) of these soaps added to 2 c.c. will effect complete solution of a 5 per cent. suspension of ox corpuscles.

Like the soap fraction of blood or organs, all these soaps become inactive when mixed with a certain amount of serum. This inactivation is again an apparent one, because the presence of suitable immune bodies hinders the paralyzing action of the serum to a great extent, or such mixture may be inactive upon normal corpuscles, but active upon those which have been sensitized properly. This complementary action of the mixture of soap and serum is absent at 0° C. and disappears at 56° C., or with age. Chlorides, sulphates or acetates of calcium or barium inactivate the mixture, just as in the case of serum complement or "extract complements." This inactivation cannot be anything more than the formation of the insoluble, inactive soaps. The action of various acids and alkalies is exactly the same as in the cases of complement and "extract complements."

In this place I must not omit reference to certain interesting phenomena which I met with during the experiments on soaps as venom activators. As we have shown elsewhere, venom is inactive without the aid of a second substance. We found that this second substance can be the complement of serum. Kyes discovered later that lecithin is activating for venom, and thinks this is the only class of bodies which is responsible for venom hemolysis. Complement has been placed in a doubtful position as a venom activator. My present work, however, again upholds our previous view that complement is a very important venom activator of serum. Certain fresh serums contain venom activator. If we add to such serums certain amounts of calcium chloride, their activating property is easily destroyed. Complement also disappears in these instances. But if we heat the inactivated serums to 75° C. or higher, then they acquire a new, powerful, venom activating property, which cannot be removed by calcium chloride. On the other hand, ox serum contains almost no venom activator in the fresh state, but acquires one when heated to 75° C. or higher, and this acquired activator cannot be inactivated by calcium chloride. If we take two tubes of fresh ox serum and add soap to one and lecithin to the other, we get venom hemolysis in both tubes. But if, before we add venom, we introduce a certain amount of calcium chloride into each tube, and then venom, venom hemolysis will occur in the tube with lecithin, but not in the tube with soap. It would be

very interesting to ascertain to what extent lecithin is concerned in venom lysis caused by fresh serum.

35 (178)

Effects of experimental injuries of the pancreas.

By **ISAAC LEVIN.**

[*From the Department of Pathology of Columbia University, at the College of Physicians and Surgeons.*]

A review of the experimental work done so far shows clearly that injuries of the pancreas produce different effects on the organism than the complete removal of the organ. After the latter operation the animal succumbs with the symptoms of subacute diabetes, but a comparatively slight injury to the organ may kill it within twenty-four hours, producing an entirely different symptom complex.

It seems very difficult to form a correct idea of the etiological relation between a certain injury to the pancreas and the disease process that so rapidly kills the animal, because in all the experimental work thus far reported, an injury which results fatally in a certain number of animals, produces no effects on others.

Doberauer reported (in *Centralbl. für Chir.*, Nr. 28, 1906) a series of twenty-one experiments on dogs. In each case he doubly ligated and severed the pancreas with identical results in all the experiments, viz., the development of fat necrosis, sub-serous peritoneal hemorrhages and free hemorrhagic fluid in the peritoneum. The animals were either dead or moribund within twenty-four hours. The author ascribes the fatal results in his experiments to a combination of stasis of secretion, some abnormality in the circulation and a lesion of the parenchyma of the pancreas. The experiments of Doberauer differ from all previous investigations in the fact that he obtained the same results in every experiment. It seemed advisable to repeat his experiments, because, if found correct, they could subsequently be varied so as to afford a clearer insight into the etiological moment of the injury which produced the acute fatal disease of the animal.

The operation of Doberauer was first repeated in exactly the