ing to a maximum soon after sulfonylurea administration(11,12,13).

It has been postulated that any insulin-like substance released from the pancreas would first reach the liver and exert its major effect at that point, not escaping in sufficient amount to exert any effect at the periphery(14). However, the present study has shown that the rapid injection of insulin intraportally produced effects equal to those of the same dose given subcutaneously. Moreover, despite a large number of investigations the existence or exact nature of a direct insulin effect on the liver remains controversial(15).

The hypothesis that these agents act by release of pancreatic insulin is rendered more attractive by the numerous studies showing them to be ineffective in depancreatized or fully alloxanized animals. However, it is possible to obtain hypoglycemia with sulfonylureas in the absence of the pancreas if the animals are given maintenance amounts of insulin(16,17) indicating that insulin apparently exerts a permissive effect. That is, by virtue of its effect on sugar transport as well as through its other metabolic effects it provides an environment in which these agents may exert their hypoglycemic action.

Summary. Insulin markedly increased the volume of distribution of L-arabinose and D-xylose and accelerated the disappearance of D-galactose and 2-deoxy-D-glucose from the blood in functionally nephrectomized rats. Tolbutamide even in a dose in excess of that necessary to produce marked hypoglycemia was without effect on these sugars. In addition, tolbutamide did not potentiate the effect of suboptimal nor optimal doses of insulin upon the volume distribution of L-arabinose.

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Cellular Damage by Soluble Antigen-Antibody Complexes in Tissue Culture.* (29074)

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Recently, biological action of certain soluble antigen-antibody complexes formed in antigen excess has received increasing attention(1-5); these observations have suggested that the anaphylactic injury not always requires the actual interaction of antigen and

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antibody in the tissue or cell and that the injury may also be produced by preformed antigen-antibody complexes on susceptible cells. Most of such investigations were performed with serum protein antigens which permitted quantitative determination of the antigens and antibodies involved, the *in vivo* behavior of the complexes, and the physicochemical properties of the complexes.

By use of a special culture chamber which allows a parallel morphological and biochemical study of the cell, we have clarified the basic events occurring during the cellular antigen-antibody reaction; the reaction takes place primarily in the cellular membrane and cytoplasmic ground substance, simultaneously resulting in release of a sulfhydryl-dependent protease; the reaction affects secondarily the mitochondria, resulting in release of a protease inhibitor of polypeptide nature(6-8). Although such cellular damage was compared in both tissue-monocytes and fibroblasts from adult rabbits, an essential difference was not observed(9). Such events in the cell seemed responsible for anaphylactic injury such as the cutaneous Arthus phenomenon(10,11).

Through these and other(12) experiments, we proved that tissue culture is a very useful tool in basic study of tissue injury. This paper describes morphological changes in the cultured fibroblasts by soluble antigen-antibody complexes; the possible mechanism of this cellular damage is discussed.

Materials and methods. Details of the methods for cultivation have been published (6,9). Explants 1 mm diam. were prepared from the omentum of albino rabbits (2-2.3 kg). Medium fluid was composed of horse serum, 5% lactalbumin hydrolysate (N.B.C.) in Gey's buffered solution, and Gey's in ratios of 1, 1 and 8, and adjusted to pH 7.2. Our culture chamber(6,7) was used. In general, 4 explants were placed on the inner surface of the small round coverslip, without using plasma, then medium fluid was introduced. The chamber was tighty closed and sealed on the outside with melted paraffin. Medium fluid was renewed every other day with a tuberculin syringe attached by Dunlop tubing to the inlet of the chamber. After 5-6 days, almost every cell in each culture was a fibroblast. Observation of cultured cells was made with a Tiyoda's phase contrast microscope.

Soluble BSA-rabbit anti-BSA complexes used were prepared by Dr. K. Ishizaka, Nat. Inst. of Health, Tokyo(4) and generously supplied. The complement fixability to the soluble complexes was determined by him following the method of Mayer *et al*(13). Two milliliters of the test samples, adequately diluted, were added to 400 C'_{H50} units of complement in a final volume of 4 ml after 19 hours at 4°C. The residual activity of complement in terms of C'_{H50} was determined.

Results. Each complex was diluted with 5, 10, 50 and 100-fold volume of medium fluid at use; and 0.7 ml of each solution was introduced into the culture chamber. Although there was slight variation, cellular changes by 5- and 10-fold diluted complexes were intense, but those by 100-fold diluted complexes were apparently less intensive. Earliest visible changes occurred in the form of accelerated motion of many minute pseudopods or membranous pseudopods, the changes becoming evident 2-5 minutes after application of the complexes. Although the cells did not normally show pinocytosis, small pinocytotic vacuoles appeared and were ingested within the cytoplasm through active folds of undulating membranous pseudopods. Mitochondria, filamentous or rod-shaped, also became active and moved to and fro in the cytoplasm. Such early changes in the cells seemed to result from cellular excitation due to the action of complexes. Ten to fifteen minutes after addition of the complexes, the pseudopods decreased in length but increased in thickness, as they began to retract. Twenty to thirty minutes after addition of the complexes, pinocytosis seemed to reach its peak, accompanied by active undulation of membranous pseudopods. Some of the pinocytotic vacuoles fused to each other, or disappeared gradually; some migrated toward the peri-Golgian area and then disappeared in the area. During this period, most of minute pseudopods were fused with neighboring pseudopods or retracted; retraction of larger pseudopods increased in intensity, the cells be-

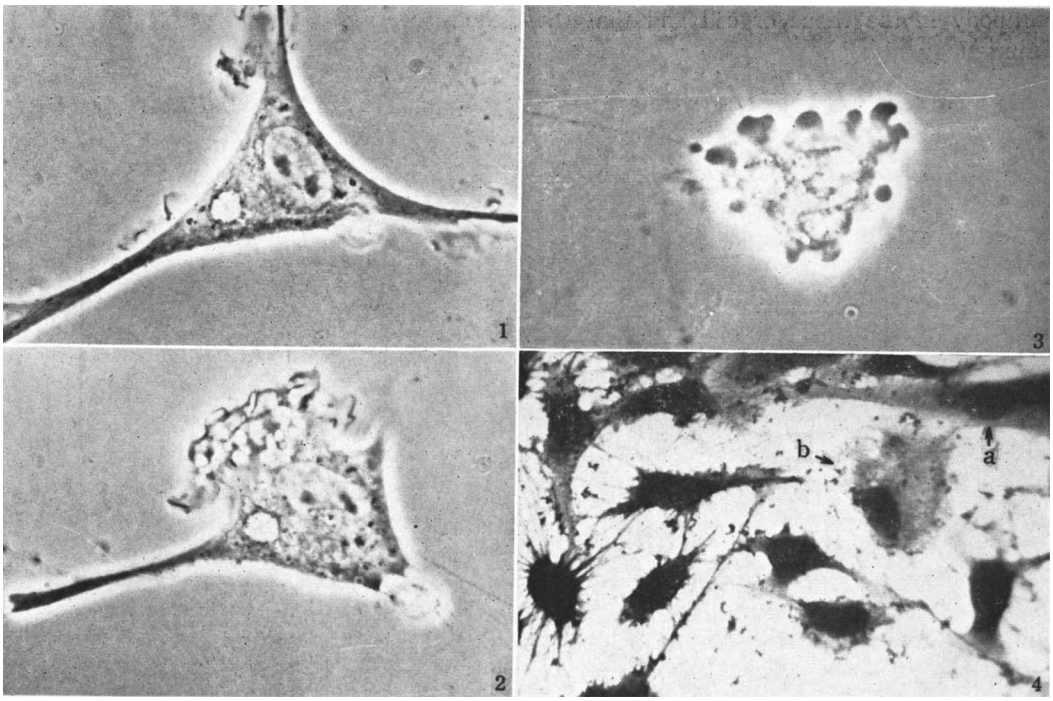

FIG. 1. Cultured fibroblast before contact with complex A (5-fold dilution). × 975.

FIG. 2. The same cell, 25 min after addition of the complex. Note active pinocytosis and retraction of larger pseudopods. Mitochondria filamentous . × 975.

FIG. 3. The same cell, 50 min after the complex. Note active blister formation. Mitochondria granular, nucleus in shrinkage. Cell in spherical deformation. × 975.

FIG. 4. Stained with hematoxylin and eosin. 50 min after the complex. a: non-injured cell. b: injured but not completed cell. All other cells showing completion of cellular changes. × 260.

coming gradually spherical. No change was yet observed in mitochondria and nuclei, though mitochondrial movement became less active. Forty to fifty minutes after addition of the complexes, pinocytosis was apparently reduced, the cells formed blisters of varying size, the mitochondria became granular or vesicular, and the nuclei showed varying degrees of shrinkage. No injury to the cells was produced with antibody alone or antigen alone throughout 6 hours. The amount of antibody and antigen added corresponded to the amount (total N mg) of the soluble complexes.

Time-course of the cellular changes was varied in individual cells, but the changes always were completed within 80-90 minutes after the complexes. Severe damage occurred in 58-64% of cultured cells when complex A and B were applied, but in 40% when complex C was applied in similar conditions (5-fold diluted complexes). The time-course in the damage seemed related to the intensity of pinocytosis; the more intense the pinocytosis, the faster was the completion of cellular changes. Since the cells during mitosis did not usually display pinocytosis, it was reasonable that mitotic cells showed no such cellular changes. After completing the mitotic processes, each divided cell was damaged as described above. At various intervals after the complexes, the cells were fixed and stained routinely. The injured cells were characterized by spherical deformation, pyknosis of the nuclei, eosinophilic staining of the cytoplasm and formation of many hair-like processes. By these features the injured cells could be easily differentiated from non-injured cells.

Discussion. Cellular changes just described were clearly initiated following characteristic cellular excitation, that is, in the form of accelerated motion of pseudopods and increased pinocytosis, possibly due to some

TABLE I. Properties of Soluble BSA-rabbit Anti-BSA Complexes.

Complex	Total N (mg)	Ab N (mg)	Ag/Ab (wt)	C'_{H50} units fixed μg Ab N*	Skin† reaction	Injured cells‡ (%)
A	8.7	3.76	1.31	6.2	++	64.1 ± 12.0
B	7.1	1.58	3.7	5.2	++	58.2 ± 11.2
C	5.08	.8	5.35	1.4	+	40.4 ± 9.81

* Complement fixability determined by Dr. K. Ishizaka.

† Maximal skin reaction 3 hr old. 0.1 ml of 5-fold diluted complexes intradermally injected in depilated flanks of adult rabbits. Reaction graded following the previous method(14).

‡ 5-fold diluted complexes applied. Counted for 2000 cells of each culture.

functional change of cellular membrane activity by the complexes. Such change of the membrane seemed responsible for the cellular damage. Delayed changes affecting mitochondria and nuclei, in part, seemed related with the complexes ingested through increased pinocytosis. Some workers(15,16) have pointed out a definite relationship between the fluid (protein) droplets of pinocytosis and the filamentous mitochondria. Other workers(17,18) have shown that the complexes agglutinated thrombocytes but the function of the agglutinated cells was not altered.

Present experiments show characteristic basic patterns of cellular damage by the complexes. Such descriptions do not appear to have been previously reported. The data also indicate that injurious effects of the complexes on the cell or tissue (skin) may be correlated with their complement fixability (Table I).

It was interesting that cellular damage by the complexes resembled that in the antigen-antibody reaction in sensitized fibroblasts. The cellular changes in the latter reaction also occurred in about 60% of cultured fibroblasts, and were completed about 60-70 minutes after the homologous antigen (2.0% BSA). However, similar changes occurred in about 95% of cultured tissue-monocytes. Accordingly, it seems probable that cellular damage described here may play an important role for tissue lesion by the complexes. It was also interesting that in the antigen-antibody reaction, features of cellular excitation as noted above were not evident. There occurred rapid fusion or retraction of minute pseudopods and rapid stopping of undulation of membranous pseudopods; pinocytosis was apparently less intense. Our previous work (19) suggested the irreversible change of cellular membrane was due to the actual interaction of antigen (Congo red-azo-bovine serum albumin) and antibody arranged in the membrane. Such difference in early reactions is being studied by means of phase contrast-cinematograph.

Summary. By tissue culture method and with the use of phase contrast microscope, detailed morphology of cellular damage by soluble BSA-rabbit anti-BSA complexes was studied. Although the changes resembled those in the antigen-antibody reaction, they were characterized by early cellular excitation accompanied by increased pinocytosis. Injurious effects of the complexes seemed associated with the complement fixability of the complexes.

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Island-Cell Tumors in Irradiated Rats in Parabiosis.* (29075)

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The incidence, function and age and sex distribution of adenomas and carcinomas of the islands of Langerhans were studied in Deac-Slonaker rats. These lesions are very rare in rats, and we have not encountered them in normal control rats of this or other strains. In the course of experiments to determine the effects of a single supralethal dose of radiation on duration of life and incidence of tumors we parabiosed young inbred littermate rats of this strain. The technique, described elsewhere(1), was to irradiate one of the pair some weeks after parabiosis and when equilibrium had been established between the animals. The right-hand rat received a single supralethal dose of 1000 r of 250 KV X-rays. The left-hand rat was shielded. The LD_{50} for single rats is 750 r. Parabiosis protects the supralethally irradiated animal from death and from the most marked effects of the acute radiation syndrome. Parabiosis in itself provides an abnormal hormonal balance in the animals, which is accentuated by the irradiation of one. We found tumors of the islands of Langerhans rarely in control parabiotic rats, commonly in the irradiated partner. The animals were allowed to live until dead or moribund.

For this study 715 pairs of parabiont rats were chosen at random, but with life spans of over 200 days after operation or irradiation. One hundred thirteen pairs of these parabiosed animals, approximately equally divided as to sex, were used as controls. Of the irradiated pairs 276 were male to male parabionts, 326 female to female. The findings are set out in Tables I and II. Males are strikingly prone to develop these pancreatic tumors.

Morphology. The tumors of the islands are usually large enough to be seen grossly (0.4 to 1.2 cm). The appearance of the benign tumors was fairly uniform, and they could be separated morphologically into two types.

The first type closely followed the architecture and vascularization of the normal island, notwithstanding the distortion caused by the growth process. The central portion maintains an orderly arrangement with short and narrow cell cords; the periphery is less orderly and occasional "blood lakes" are present. Capsule formation may be incomplete. Some lesions retain alpha cells along with the dominant beta cells. These adenomas perhaps could be classed as "giant islands."

The second type more obviously meets the neoplastic criteria of Borst(2): it shows marked structural variations from the normal

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