on insulin response compared to that obtained in buffer medium alone may be due to its protective action in preventing loss of insulin by adsorption or degradation.

Comparison of "serum insulin" levels in normal animals have shown that there is a considerable range among normal individual animals; normal rats, for example, have shown as much as a 1000-fold variation in serum activity. Although some of this scatter may be a reflection of the inherent variability of the bioassay technic employed, results so far lend support to the view that significant changes of "serum insulin" occur under normal physiologic conditions. The most interesting observation in comparing relative activities in the various animal species was the marked difference between human and rat serum insulin-like activity. The order of increasing activity found was: human<mouse, rabbit, dog<<rat. Because of its significantly higher "serum insulin" level, rat serum should be particularly useful in studies to characterize the serum constituent which stimulates glucose uptake and to establish its relationship with "serum insulin."

The general shape of the insulin clearance curve in rabbits (Fig. 4) is similar to that obtained by Scott and co-workers(5) who employed a higher dose (15 units/kg) than the 1 unit/kg dose used in our studies. Although they found a 6-fold maximum increase in insulin concentration over pre-injection level, we have found over a 40-fold increase while employing 1/15 the amount of injected insulin. This difference may be explained in part of the much higher value of 2.5 milliunits/ml they report for normal rabbits before injection compared to 0.08 milliunit/ml which we have found using the mouse diaphragm technic.

Summary. The mouse diaphragm technic has been adapted to measure insulin-like activity of dialyzed serum. Compared to the response effected by insulin in KRB buffer medium alone, normal rabbit serum inhibited while beef serum albumin enhanced insulin response. Mean serum insulin level of adult rats was significantly greater than rabbits, mice, and dogs and much higher than humans. A significant elevation in serum insulin activity which persisted up to 45 minutes following injection, was found in rabbits injected intravenously with 1 unit/kg insulin.

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Immune Electron Microscopy. (25827)

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In the past decade advances in the study of immune reactions of bacterial, viral, and mycotic agents have been made, using immunofluorescent technics. It has become apparent that even more refined technics are desirable because of the low resolution with this technic, to prove that in some systems the reactions are specific and not due to nonspecific uptake of the fluorescent dye, and also to provide a means of studying pathogenesis at a subcellular level. Singer(1) prepared an electron-dense conjugate by coupling ferritin to an antibody, indicating that specificity of the antibody was probably not altered. Using

his concept, an immune serum to *Staphylococcus aureus* was conjugated to ferritin by a slightly modified method, to serve as a model for study of immune reactions at the subcellular level.

Materials and methods. To 10 ml (0.5 g) of horse spleen ferritin was added 20 ml of 0.5 M carbonate-bicarbonate buffer, pH 9.0, at 4°C. With slow stirring, to prevent frothing, 0.6 ml of m-xylylene diisocyanate was added dropwise and stirring was continued for three-quarters of an hour at 4°C. The supernatant, the ferritin conjugate (FeIso), was removed by centrifugation and was allowed to stand in the cold for 1 hour. An immune serum to *Staphylococcus aureus,* Type I, (ATCC 12598) was prepared from a boiled broth culture according to Hobbs(2). The serum was diluted with physiologic saline to contain 10 mg protein/ml, 10% of total volume being the carbonate buffer. At 4°C an equal volume of FeIso conjugate was added dropwise with slow stirring, and the stirring continued for 2 days. The mixture was dialyzed in the cold for 5 hours against 0.1 M ammonium carbonate and overnight against 0.1 M phosphate buffer, pH 7.2. A clear, brownish conjugate was obtained after centrifugation. The preimmunization serum was similarly treated to serve as a control. An 18-hour agar culture of the homologous organism was fixed in 10% buffered formalin and washed in buffered saline. The suspension was divided into 2 aliquots. One aliquot was allowed to react with the immune conjugate, the other with the control conjugate. Both were washed, run through graded alcohols, and embedded in methacrylate with uranium(3). Thin sections were cut and placed on a grid and observed with the RCA EMU 3D electron microscope with a 1 mil objective aperture at initial magnification of 9,000 for Fig. 1 and 2. Fig. 2 insert was taken at an initial magnification of 30,000.

Results. The ferritin-antibody conjugate retained its specificity, and antigen-antibody reaction was easily detected by the characteristic pattern of the ferritin molecule. The ferritin was completely washed out of the background, preventing confusion with a specific reaction. The control (Fig. 1) shows the capsule-like material as being a homogeneous entity, while the test with immune serum (Fig. 2) shows ferritin molecules in the capsule-like material, indicating an antigen-antibody reaction. Fig. 2 (insert) shows the molecular structure of ferritin as described by

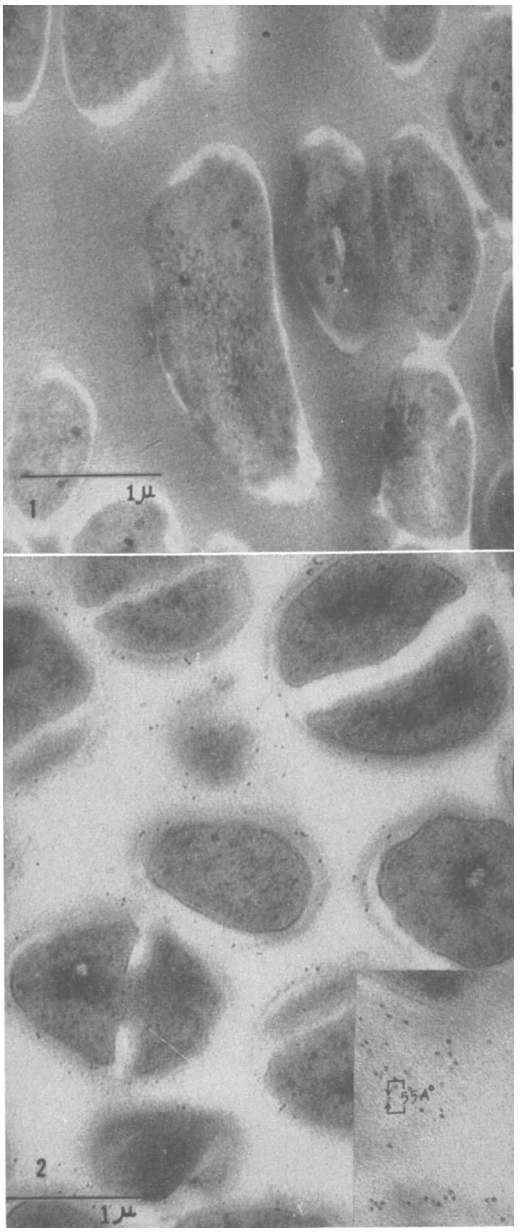

FIG. 1 and 2. Sections of *Staphylococcus aureus* reacted with (1) control conjugate; (2) immune conjugate; (insert) higher magnification showing ferritin molecule.

Farrant(4), in the capsule. In the sections of the organisms in mitosis an occasional spindle-like structure can be observed, as well as what appears to be a double membrane at points of division.

Discussion. Use of electron-dense antibody conjugates presents a new method of observing immune reactions at subcellular level in formalin-fixed preparations. Heretofore formalin fixation has been used infrequently in electron microscopy, as it was believed to reduce ability to obtain good resolution of cellular material. However, the electron-dense material, in what is considered as nuclear region, is well demonstrated in our sections. It has the further advantage of rapidly inactivating any infectious agents used. Although formalin fixation may not be as satisfactory for other tissues as it apparently is for staphylococci, it appears warranted to further investigate its usage in electron microscopy. As unbound ferritin conjugate was completely removed by simple washing, the problem of nonspecific reactions with the immunofluorescent technic was not encountered. This will permit a more critical evaluation of specificity of immune reactions, a problem which has been most troublesome in formalin-fixed tissues. It should now be possible to localize definitely the sites of antigen production as well as viral aggregates in tissues that have been properly fixed with formalin as well as other material at the subcellular level. The number of ferritin molecules around bacteria could probably be increased by changing ratio of antigen-antibody in the reaction.

Summary. A method is described by which immune systems can be studied at subcellular level with the electron microscope, using a ferritin-antibody conjugate. A conjugated immune serum to *Staphylococcus aureus* was applied to homologous organisms, embedded in methacrylate with uranium and thin sections cut. An antigen-antibody reaction was detected by attachment of the ferritin molecule in the capsule-like material. Use of formalin in primary fixation for electron microscopy as well as nonspecific immunofluorescence is discussed.

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Effect of 5-Hydroxytryptamine on Phosphorylase and Glycogen Levels in Muscle Tissue.* (25828)

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Although the isolated uterus of the estrous rat is frequently employed for bio-assay of certain psychopharmacological agents and hormones, little is known concerning the effects of these substances, particularly the former, on glycogen stores and enzyme phosphorylase in uterine muscle. Epinephrine and oxytocin, however, induce a rapid decrease in both uterine glycogen(1) and phosphorylase activity(2). The effects of serotonin (5-OH-tryptamine), lysergic acid and reserpine on these 2 constituents in uterine smooth muscle were investigated. Since serotonin alone produced changes in smooth muscle, its effect was studied also on skeletal, cardiac, and diaphragm muscle.

Methods. Adult female rats were spayed, 7-9 days later injected with 50 μg of estradiol benzoate (Schering Co.) subcutaneously and 48-56 hours later given an intraperitoneal injection of the test compound. Uteri were removed 30-60 minutes later from the anesthetized rats and were frozen and stored as de-

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