future work to study effects of plasma and tissue levels of zinc on insulin activity.

Summary. Zinc in concentrations in the range of those observed in human plasma and higher inhibited the activity of isolated rat epididymal adipose tissue in the presence of insulin and glucose. Of the other divalent ions studied only mercuric and to a lesser extent cobalt and nickel ions inhibited insulin activity. Zinc inhibition could be prevented in part by dialysis at pH 3.75 but not 7.6. The inhibition by zinc could be prevented by EDTA or large amounts of phosphate.

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Experimental Specific Serum Therapy of Staphylococcus.* (31487)

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The recent history of the Staphylococcus problem is replete with quotations such as the following: "Among the more chastening chapters in the annals of microbiological research is the story of our apparently dismal failure to control the depredations of the Staphylococcus"(1). The seriousness of the problem has been emphasized by Rogers(3) who proposes the view that antibiotics are probably the cause of "the emergence of Staphylococci as a troublesome hospital problem." A possibility of immune therapy has been raised by Fisher and Manning(4) as they report the high content of natural antibodies in pooled human serum (See also Fisher, 5). Active immunization, with the use of improved vaccines, is shown to be feasible by McCoy and Kennedy(2). In the experiments reported below, specific immunotherapy is examined with the use of the mouse protection test.

Materials and methods. Experimental animals. Mice from the Charles River Colony were used in these experiments. The rabbits were albinos purchased from the Mission Laboratories in Los Angeles.

Vaccines. An isolate of *Staphylococcus aureus* (Ha #1) from a fulminating and fatal infection in the Los Angeles County General Hospital was obtained and the method of McCoy and Kennedy(2) was used for preparation of the vaccine. We had attempted earlier to evoke antibodies in goats with vaccine prepared by the usual pasteurization procedures, but found that even when the heat treatment was carried to the point where no growth in standard media could be detected, the vaccine still showed viable organisms upon injection into the animals. In the present series of experiments the organisms were grown for 18 hours on blood agar plates and washed off with sterile saline. After 3 washings and resuspensions of the organisms, the final suspension was adjusted to MacFarland standard tube #2 and 1 part in 7 of benzalkonium chloride (1-1,000) added. The vaccine was agitated and incubated at 37°C for 30 minutes. Following this step,

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the vaccines were tested for growth in nutrient broth. In no instance was growth observed. No evidence of infection was seen in any of the animals immunized with vaccine prepared by this method. For some of the experiments this vaccine was further processed by sonication with a Branson ultrasonic sonifier, model LS—75 for 4 hours at 6 amperes.

Preparation of antisera and globulin. The antiserum used was produced in rabbits with a Staphylococcal vaccine from an isolate termed Ha #1. A series of 13 rabbits was given weekly intravenous injections of 1 ml each of the whole cell Staphylococcus vaccine. On the fourth week, intraperitoneal inoculations of 10^5 live organisms were made and the dose increased gradually in the ensuing weeks until after 6 months the animals were tolerating 1.5×10^6 organisms. This weekly dose was continued for an additional half year. The pooled sera from these animals were stored at —20°C until used. A globulin was prepared from this serum by a slight variation of Kekwick's(6) method, using first 18%, then 14% sodium sulfate for the salting out step. A further purification was made by passing the preparation through Sephadex G-200. The second peak eluted from the column was recovered and utilized as a relatively pure IgG preparation. Another group of rabbits was immunized weekly for a period of several months with a vaccine prepared from the Ha #1 isolate, according to the method of McCoy and Kennedy, and then sonicated as described above to disrupt the cells. A goat (#37) was immunized by injection of 1.4×10^6 killed organisms (Ha #1) into the teat canal of each mammary gland a week before parturition. The colostrum was pooled, centrifuged, adjusted to pH 4.3 at 37°C and centrifuged again to remove the casein, and then salted out by Kekwick's method.

Statistical significance of the mortality records was judged with the aid of the 4-fold contingency tables of Mainland and Murray (7), a rapid method for the assessment of significance at the conventional 5% and 1% levels.

Results. The first series of experiments was designed to test the effect of immune goat gamma globulin administered intraperitoneally upon an experimental infection with Staphylococcus. Nine mice in the experimental series were injected with gamma globulin from the colostrum of goat #37. Each received 30 mg of the globulin in 2 ml of saline, i.p., 23 hours before challenge and 15 mg in 2 ml of saline 5 hours before the challenge. The mice were then challenged with a washed suspension of approximately 10^9 live *S. aureus* intravenously *via* a tail vein. Concentrations of organisms were adjusted with the aid of MacFarland tubes. Nine animals serving as controls were given the same dose, 10^9 *S. aureus* organisms in a tail vein without other treatment. Fig. 1 shows the mortality curve of the 2 groups. The protection shown in those receiving the gamma globulin was significant at the 5% level of confidence between hours 42 to 65. In the experiments where the passive immunity was not continued the mortality of the experimental group then approached that of the controls.

A second set of experiments tested the effect of oral administration of highly immune rabbit globulin on an *S. aureus* infection. The mice were given the globulin dissolved in their drinking water for 5 days prior to the challenge. The dilution was adjusted so that the average intake of each mouse was 30 mg per day. The same dose was fed the animals in the 24 hours following the challenge; however, the mice drank practically none of the solution after the organisms were administered. As controls, a group of 11 mice received no globulin. All were challenged with approximately 10^9 *S. aureus* organisms in a tail vein. Fig. 2 shows the mortality curve of these experimental and control groups. Protection was shown by the delay in the mortality curve, the difference being significant at the 1% level of confidence between the 32nd and the 68th hours. Following this period of protection the experimental group approached, but did not reach, the mortality levels of the control group.

Further experiments were designed to test the protection offered by feeding a smaller dose of the same rabbit globulin which had been further purified by passage through a Sephadex G-200 chromographic column. For

comparison a similar group of animals was fed the whole globulin and a control group (n = 13) received no protection. The two experimental groups (n = 12, 12) were fed 45 mg of their respective globulin preparations in water over a period of 48 hours prior to the challenge. The mice were challenged with 10^9 *S. aureus* organisms injected into a tail vein. Mortality curves of the 3 groups (Fig. 3) illustrate that the 7S globulin showed more protection than the whole globulin. Between the 50th and 70th hours the difference between the first group and the controls was significant at the 5% level of confidence.

In another series of experiments the specific globulin was given orally or intraperitoneally to compare the protective effect of these different routes of administration. Four test groups received protective doses as follows: 5 mg and 20 mg intraperitoneally, and 100 mg and 200 mg orally. The oral globulins were administered in 5 daily doses. The injected groups were given the globulin 5 hours before challenge. All mice were then injected

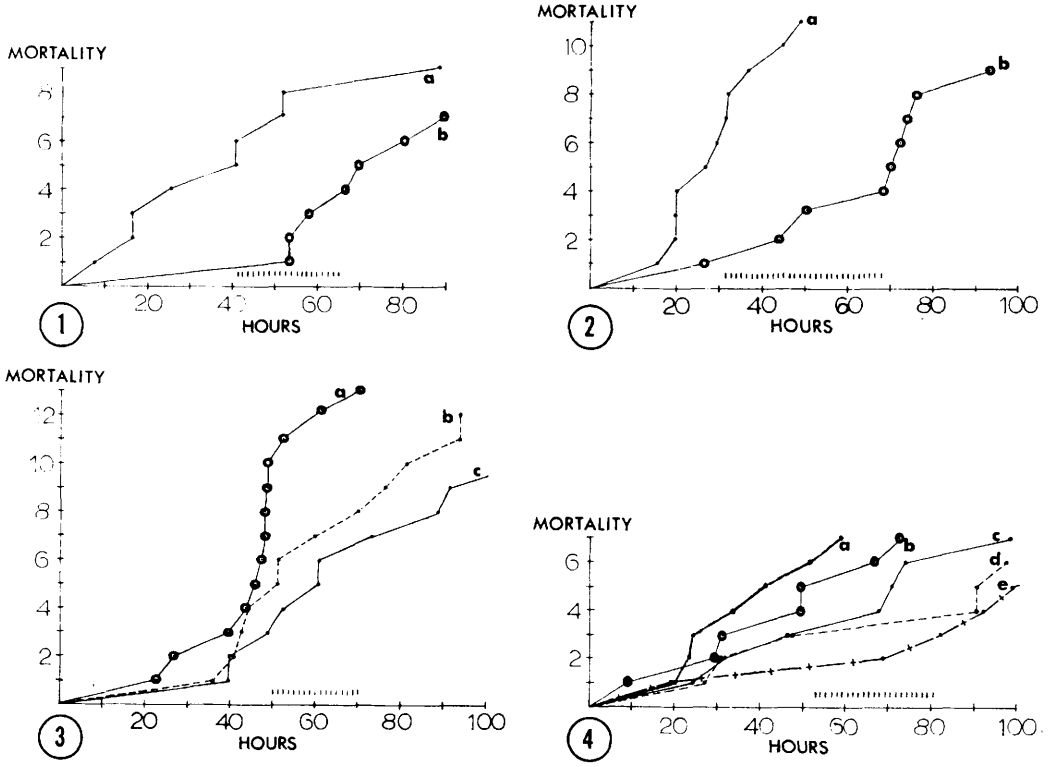

FIG. 1. Mortality curves for (a) control mice injected i.v. with 10^9 *S. aureus* and (b) experimental group injected i.p. with 45 mg gamma globulin per mouse, from goat immunized to same organism and then subjected to same challenge as in (a). Interrupted bar denotes period during which protection was shown at the 5% level of confidence.

FIG. 2. Mortality curves for (a) control mice injected i.v. with 10^9 *S. aureus* and (b) mice given 5 daily doses by mouth of 30 mg gamma globulin per mouse, from rabbits immunized with same organism and then subjected to same challenge as in (a). The interrupted bar denotes period during which protection was shown at 1% level of confidence.

FIG. 3. Mortality curves of (a) control mice (n = 13) injected i.v. with 10^9 *S. aureus*, experimental groups (b) and (c) (n = 12, 12) given 45 mg orally of whole rabbit globulin and isolated gamma globulin respectively, over a period of 48 hours, following which they were subjected to the same challenge as (a). The interrupted bar denotes the period during which the protection shown in group (c) was shown at 5% level of confidence.

FIG. 4. Mortality curves for (a) control mice receiving 10^9 *S. aureus* i.v. and (b - e) groups similarly challenged after administration of immune globulin from rabbits immunized with the same organisms. Groups (c, e) fed 100 mg and 200 mg, respectively, administered in 5 daily doses. Groups (b, d) injected i.p. 5 hr before challenge with 10 mg and 20 mg respectively.

via a tail vein with 10^9 live *S. aureus* organisms. Fig. 4 illustrates the mortality curve of these 4 groups and their controls. Between the 53rd and 80th hours the difference in mortality of the control and of the group receiving 200 mg gamma globulin orally was significant at the 5% level of confidence. Comparison of the 4 experimental groups shows that the ratio of effectiveness of the intraperitoneal injection over the orally administered gamma globulin was of the order of about 10 to 1.

A series of rabbit experiments was carried out to test the effect of protective globulin upon local infections with *S. aureus*. Ten rabbits were divided into equal groups. Three globulin preparations were tested by the following experiments. Two rabbits were given 10 ml of rabbit serum from the series of animals immunized with a vaccine in which the organisms were broken up by sonication. Another group was injected with 10 ml of whole pooled rabbit serum obtained from animals immunized with the benzalkonium-killed whole organism vaccine. The third group was given 300 mg of goat colostrum globulin from goat #37, immunized *via* the teat canal with the benzalkonium-killed whole organism vaccine. The fourth group was immunized during the 4 weeks prior to challenge in the following way: bi-weekly injections of

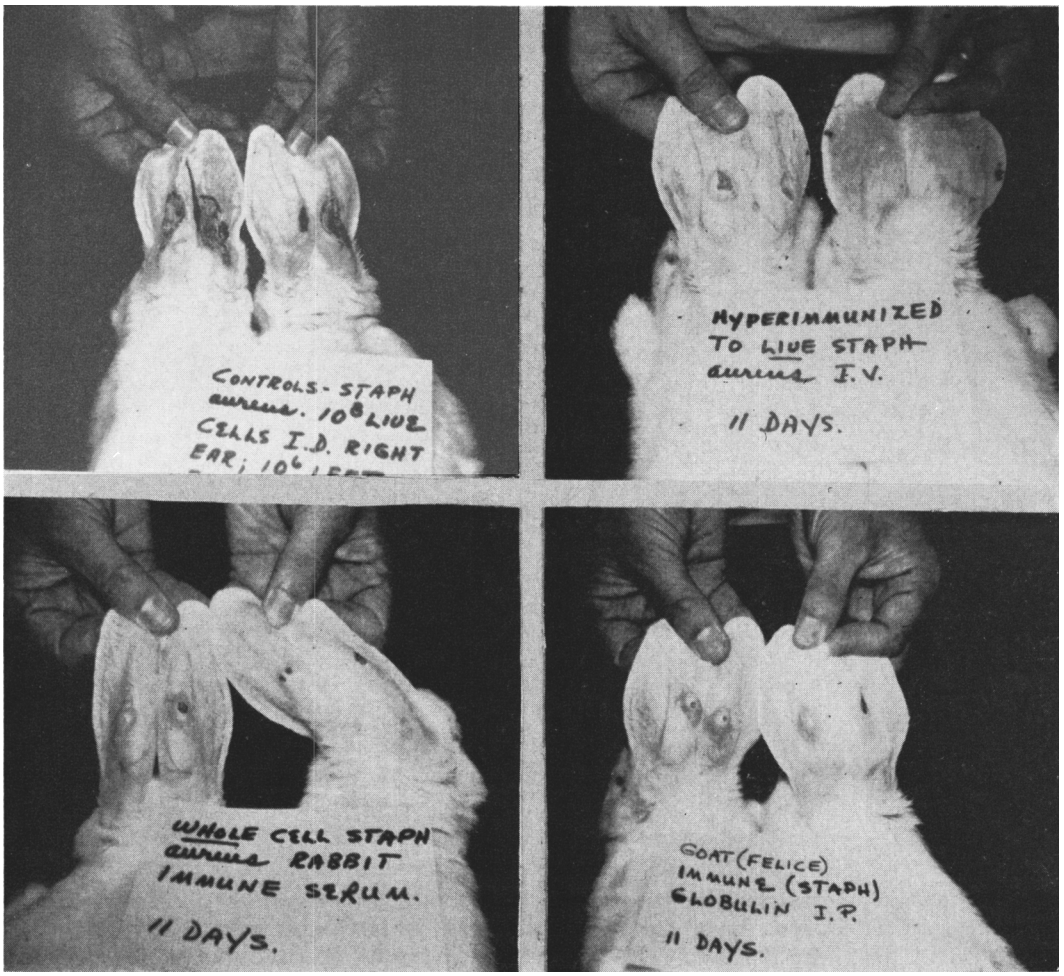

FIG. 5. Photographs to show ears of animals injected subcutaneously with 10^6 *S. aureus* organisms, in each left ear, 10^8 in the right. (a) control, (b) protected by active immunization (see text), (c) given immune rabbit serum, and (d) given goat colostrum globulin. All photos taken on 11th day.

killed *S. aureus* were made intravenously for the first 2 weeks and small doses of live organisms were injected i.v. for the second 2 weeks. These 10 rabbits were challenged with a live 18-hour culture of *S. aureus* (Ha #1) from blood agar plates, washed 3 times in sterile saline. Injections in volumes of 0.1 ml were made in the posterior surface of the ear subcutaneously with the number of organisms adjusted so that each animal received 10^6 cells in the left ear and 10^8 cells in the right ear. The animals were watched daily and the lesions were photographed at 1, 4 and 11 days. The controls showed severe reaction at 1 day with necrosis of tissue and sloughing of skin. This reaction and sloughing was evident through the 11th day and resulted, weeks later, in large fenestrations through the ear. In contrast, all of the protected animals showed much less reaction with little or no necrosis and well healed lesions at 11 days. The animals which had been actively immunized with the live and killed organisms showed better protection than those in the other treated groups.

Discussion. The ubiquity of pathogenic Staphylococcus means that nearly all animals are highly immune to these organisms. The high degree of immunity of the experimental animals means that very large doses of the infectious organisms must be given. At the dose levels which were necessary in these experiments, the problems of exotoxins and of absorption of antibodies become troublesome, making it essential to wash the infectious organisms very carefully to prevent carry over of exotoxins and early death of all the animals. In order to overcome the immunity of the animals, it was necessary to give them large doses of the organisms. Therefore, to demonstrate the effect of globulin, it was necessary to give what might seen to be excessive amounts of protective antibody. It was our purpose here to show, that in spite of the unfavorable factors involved, experiments could be performed to test the protective effects of immune globulin.

We have experimented with a mild method of producing vaccines in an effort to utilize as much of the antigenic structure of the bacteria as possible. The production of antitoxins has been minimized by washing the Staphylococcus suspensions thoroughly. The observations of Quie and Wannamaker(8) that cellular bactericidal mechanisms are necessary for defense against Staphylococcal disease, have led us to direct our efforts toward the antibacterial systems.

The benzalkonium-killed vaccine made according to the method of McCoy and Kennedy(2) proved superior to any others which we have used. Excessive heat treatment is necessary to insure sterility of vaccine and so lessens its value for immunization. With the benzalkonium treatment, however, this can be avoided, and 100% sterility of the vaccine is obtained.

From these experiments it was seen that the oral route of administration requires approximately 10 times as much antibody as the intraperitoneal route. This result agrees with data reported previously(9) on a similar comparison in mice utilizing *Salmonella pullorum* as the infectious organism. In the experiments described here the antibody was given to the mice in their source of fluid, while those previously reported utilized the method of gastric intubation for delivery of the therapeutic globulin. The ratio of effectiveness of oral *vs* intraperitoneally injected globulin seems not to have been determined in other species.

For practical immune therapy against Staphylococcus disease a necessity exists for new sources of antibody, new methods of administration of antibody, better methods of proving the protection of therapeutic preparations, and practical methods of avoiding serum sickness.

Summary. Protection at significant levels was shown in experimental mouse infection with *Staphylococcus aureus* by globulins from immune rabbit blood serum and from goat colostrum. Immunizations to produce the protection were made with benzalkonium-killed organisms alone, or followed by injections of live organisms. Both the intraperitoneal and oral route of protection proved efficacious in the mice. Experimental subcutaneous abscesses in rabbits were similarly studied and the suppression of inflammation and necrosis shown.

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In vitro Inhibition of Intestinal Fluid and Electrolyte Transfer by a Non-Beta Islet Cell Tumor. (31488)

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Zollinger and Ellison(1) described a syndrome of intractable peptic ulceration and gastric hypersecretion associated with non-Beta islet cell pancreatic neoplasms. Subsequently, Verner and Morrison(2) pointed out that non-Beta islet cell tumors may also be associated with profuse watery diarrhea and hypokalemia without gastric hypersecretion or peptic ulceration. Although the ulcerogenic mechanism has been partially clarified by isolation of a gastrin-like substance from these neoplasms(3), the diarrheogenic mechanism remains obscure. This report presents evidence which suggests that the diarrhea in a patient with a non-Beta islet cell tumor of the pancreas resulted from the tumor or its products inhibiting the net intestinal absorption of fluid and electrolytes.

The patient who stimulated this study was a 31-year-old male with a non-Beta islet cell pancreatic adenocarcinoma, intractable diarrhea (15-40 bowel movements per day with stool volumes as high as 8 liters per 24 hours) and hypokalemia without peptic ulceration or gastric hypersecretion. Balance studies performed on this patient demonstrated excessive fecal losses of fluid and electrolytes. Mouth-to-anus transit time measured with an indigo carmine marker on 2 occasions was normal. Continuous aspiration from the patient's distal ileum demonstrated a pronounced increase in the volume of fluid passing from the small intestine into the colon. These excessive volumes were obtained during continuous simultaneous gastric aspiration, thus excluding a significant gastric contribution to the excessive ileal fluid. The clinical studies will be reported in detail later.

Materials and methods. Liver metastases, histologically proven to be a non-Beta islet cell adenocarcinoma of the pancreas, were obtained at laparotomy and immediately frozen. Assay of the tumor for gastrin-like activity was negative.* A sample of the patient's serum, an autopsy specimen of histologically normal liver tissue and pancreatic tissue from a patient with an accidentally ligated pancreatic duct were also obtained and frozen. Prior to each experiment, the tumor, liver and pancreatic tissue were thawed, homogenized and extracted with distilled water such that the extracts contained 100 mg of tissue per ml of distilled water.

Fluid and electrolyte transfer: Experiments were carried out on adult, male Golden Syrian hamsters (210-240 g) maintained on a commercial diet, (Wayne Lab-blox) with unrestricted access to food and water. The animals were sacrificed and the small intestine was immediately removed and everted according to the method of Wilson and Wiseman (4). The combined jejunum and ileum was

* Kindly performed by Dr. M. I. Grossman, Veterans Admin. Center, Los Angeles, Calif.