and hamsters, without the need of syringes, needles or transferring of blood.

Materials and methods. Standard heparinized microhematocrit capillary tubes are broken into thirds. The sharp (broken) end should not have too long a bevel. With the animal held firmly or anesthetized, the sharp end of the tube is introduced into the outer or posterior canthus of the eye, under the nictitating membrane. With a slight twist, it is directed straight in, approximately perpendicular to the surface plane of the eyeball until a slight "give" is noted. Usually, the blood will flow freely and may be collected directly into a test tube. This is easily done by turning the animal over and holding the head down; the tube will remain fixed in place. Occasionally, twisting, advancing, or slightly withdrawing the capillary tube will facilitate better flow. When the desired amount of blood is collected, the tube is withdrawn and slight pressure on the eyeball is used to prevent further bleeding.

Discussion. The only difficulty encountered in this technique is occasional clotting of the blood in the capillary tube, but, even then, blood will often flow freely around the outside of the tube, which is satisfactory for most procedures. Although the collection is more convenient with the animal anesthetized, it may be done readily without anesthesia. Exsanguination of a 115-130 g rat will yield 4-6 ml of blood and in large rats more than 8 ml can be obtained without sacrificing the animals.

Serum protein analysis performed by the Weichselbaum(2) modification of Kingsley's biuret method on samples of blood obtained by this procedure did not vary significantly from specimens obtained by cardiac puncture.

Summary. A modification of a previously reported method of obtaining venous blood from small laboratory animals is described. A short piece of heparinized capillary tubing is used to puncture the orbital sinus at the posterior angle of the eye. Sufficient quantities of blood for laboratory analysis are obtained from either anesthetized or nonanesthetized animals without ill effects.

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Type of Antibody Elevated by 5-Fluoro-2-deoxyuridine and Endotoxin During the Primary Response of the Mouse.* (29135)

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The observations that gamma globulins are a heterogeneous group of plasma proteins have led to the development of methods for characterization of the individual types. It has been found that the gamma globulins with a sedimentation constant of 19 are susceptible to cleavage by sulfhydryl containing compounds such as 2-mercaptoethanol(1). This has led to the use of these agents for a rapid and convenient differentiation of the molecular character of the antibody molecule.

Recent evidence has shown that the inbred mouse, Balb strain, will respond with antibody formation following a single injection of any of several purified protein antigens(2). In addition, it was shown that drugs capable of inhibiting nucleic acid synthesis can actually increase considerably the amount of anti-protein antibody synthesized by these mice (2,3). The conditions under which immunosuppressive drugs could act as adjuvants were compared with those characteristic of the adjuvant action of bacterial endotoxins. The

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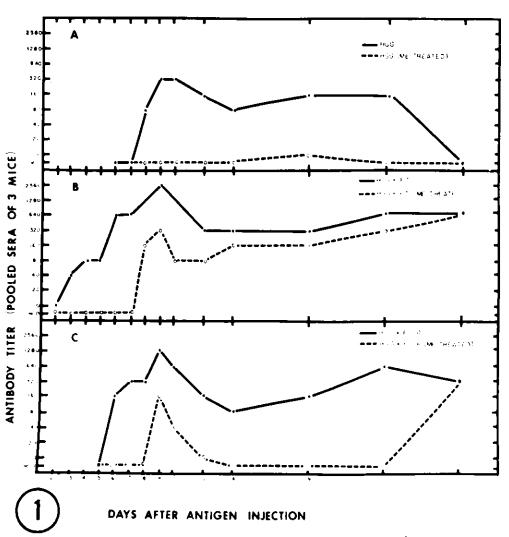
purpose of this communication is to record data on similarities and differences in the type and amount of antibody produced in mice receiving bovine or human gamma globulin under the stimulus of 5-fluoro-2-deoxyuridine (FUDR) or endotoxin (ET).

Materials and methods. Animals. Either Balb mice or C58 black mice(4) inbred in this department were used. Little variability in antibody titer between individual mice in any one group was observed. *Antigen.* Human gamma globulin (HGG) was prepared by Dr. M. Yoshioka (formerly of this department) using Cohn's cold ethanol procedure (5). Gel diffusion using a high titer rabbit anti-human plasma antiserum showed that this material contained 2 antigens. Bovine gamma globulin (BGG), California Biochemical Corp. (lot 503174), formed one line of precipitate when diffused in agar gel against a mixture of high titer anti-bovine gamma globulin and anti-bovine serum albumin antiserum.

Endotoxin. Serratia marcescens endotoxin (prepared by the Boivin procedure by Dr. A. Nowotny, Temple Univ.) was administered with the antigen intraperitoneally in a single dose of 50 μg in 0.5 ml of 0.15 M NaCl. *5-Fluoro-2-deoxyuridine* (FUDR) (Ro 50360 lot 42) was donated by Hoffmann-LaRoche Inc. and administered intraperitoneally in a single dose of 2 mg in 0.5 ml of 0.15 M NaCl 1 hour before antigen. This dose was found to give the greatest enhancement of antibody titers with no fatalities. This contrasts with the previous lot of FUDR(2) where a dose of 8 mg was found to be optimal with a fatality rate of approximately 1%. *Antibody determinations.* Mice were bled from the axillary vessels and an equal amount of blood from 3 mice pooled. Antibody titers were determined by the tanned sheep cell hemagglutination technique(6). Differentiation of types of antibody was based on sensitivity to 2-mercaptoethanol (2-ME). The observation that antibodies with a sedimentation constant between 7S and 19S were susceptible to mercaptoethanol and had characteristics between those of 7S and 19S antibody on DEAE chromatography(7) points out that the mercaptoethanol sensitive molecule need not be 19S but can be further subdivided. However, loss of antibody activity after treatment with sulfhydryl-containing compounds is considered at this time a characteristic of antibody other than the 7S type and most likely of the 19S type. In all cases the sera were diluted in a mixture of saline and 2-ME to give a 1/10 dilution of serum in 0.1 M 2-ME. Incubation for 4 hours at room temperature was then carried out and the serum diluted without removal of 2-ME for the hemagglutination test. Antibody titers of untreated sera were determined by incubating the serum in saline at room temperature for 4 hours followed by dilution for the hemagglutination test.

Results. The antibody titer formed in Balb mice following a single intraperitoneal injection of 3 mg human gamma globulin (HGG), as well as the enhancement of this response with both endotoxin and FUDR, is shown by the solid lines in parts A, B, C respectively of Fig. 1. In addition, the titer of antibody still reactive in the hemagglutination test after the sera had been treated with 2-ME is compared to the total titer in all 3 groups. In mice receiving antigen alone the total antibody synthesized within the period of 30 days was sensitive to 2-ME treatment. In the group receiving endotoxin with the antigen (Fig. 1B), 2-ME insensitive antibody began to appear on day 8. Thus the shortened induction period and elevation of peak titer induced by endotoxin appeared to be due to an enhanced early synthesis of the 2-ME sensitive antibody, assumed to be of the 19S variety. As shown in Fig. 1C, the enhancement of antibody by FUDR followed a pattern similar to that of endotoxin in that the shortening of the induction period also was due to antibody which was 2-ME sensitive.

Several additional points of interest are apparent in these data. First, a comparison of the sera obtained on day 30 in mice receiving antigen alone, antigen plus endotoxin, or antigen plus FUDR, shows that mercaptoethanol insensitive antibody (presumably 7S variety) also is increased by these 2



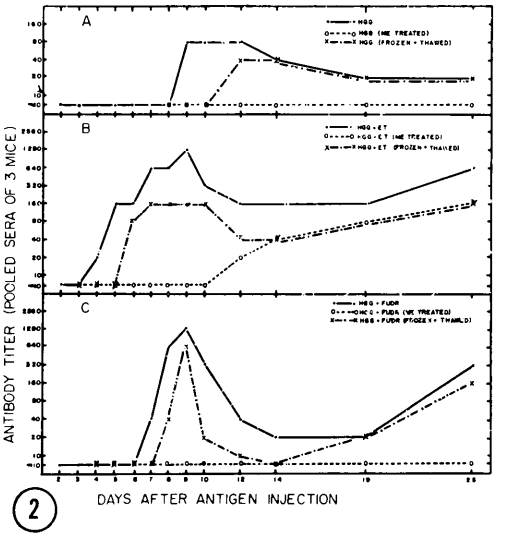


FIG. 1. Antibody titer before and after mercaptoethanol (ME) treatment of serum from Balb mice receiving *(A)* Human gamma globulin (HGG), *(B)* HGG and endotoxin (ET), and *(C)* 5-fluoro-2-deoxyuridine (FUDR) 1 hr before HGG.

FIG. 2. Susceptibility of antibody obtained from Balb mice to both mercaptoethanol (ME) cleavage and freezing and thawing. Mice received *(A)* Human gamma globulin (HGG), *(B)* HGG and endotoxin (ET), and *(C)* 5-fluoro-2-deoxyuridine (FUDR) 1 hr before HGG.

agents. Second, antibody levels as determined in the serum over a 30-day period gave evidence of a biphasic response in all 3 groups, particularly in the group receiving FUDR.

All titers in the above experiment were determined with the same reagents on the day of bleeding. The importance of determining 2-ME sensitivity on fresh mouse sera within a day of bleeding is shown by the data in Fig. 2. In this experiment which was a direct repeat of the first, the hemagglutinating antibody was determined first on serum separated from the clot after storage at 4°C overnight without freezing and thawing. In addition, a portion of each serum sample was frozen at —20°C until termination of experiment. When antibody titers were redetermined at this time on *all* the sera in each group, a drop in titer below that measured within a day of bleeding was observed. This is illustrated in all 3 groups by the dot-dash line in Fig. 2 A, B, C. The sera which were frozen and thawed were tested for 2-ME sensitivity all at the same time. It was again apparent that (a) the antibody response to HGG was comprised totally of the 2-ME sensitive antibody over the 25-day period, (b) the adjuvant action of ET was exerted on both 2-ME sensitive and insensitive antibody; however, FUDR in this instance appeared to increase only the 2-ME sensitive antibody, (c) the nature of the response of the mice to a single injection of this purified protein plus FUDR was biphasic.

One difference between the 2 experiments was the absence in the frozen and thawed serum (Fig. 2C) of the transient peak of 2-ME insensitive antibody elicited by FUDR as depicted in Fig. 1C. A second difference was an apparent delay of 4 days in time of initial appearance of 2-ME *in*sentitive antibody in the serum of endotoxin stimulated mice in the second experiment.

It was of interest to determine with a different antigen in a different strain of mouse the type of antibody increased by endotoxin. Accordingly, inbred mice, C58 strain, were given a single injection intraperitoneally of 3 mg BGG alone or together with endotoxin. When the amount of total antibody was compared to the 2-ME insensitive antibody in each of these 2 groups (Fig. 3), it was once again apparent that shortening of the induction period (which in this strain of mouse was remarkable in magnitude) was due to the synthesis of 2-ME sensitive anti-

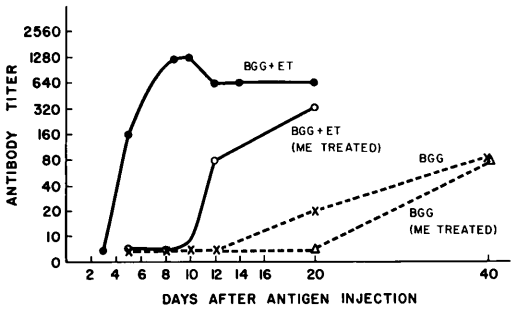


FIG. 3. Production of mercaptoethanol (ME) sensitive and insensitive antibody in C58 mice receiving bovine gamma globulin (BGG) or BGG and endotoxin (ET).

body. In addition the synthesis of 2-ME *in*sentitive antibody was stimulated to occur earlier and to a greater level by endotoxin.

Several experiments were carried out to document by more direct means that the 2-ME sensitive antibody was of a high molecular weight. Several serum samples containing only 2-ME sensitive antibody were chromatographed on DEAE cellulose using the technique of Reisner and Franklin(8). Elution of hemagglutinating activity in these samples was possible only with 0.3 M phosphate buffer pH 6. In contrast serum samples containing both 2-ME sensitive and insensitive antibody showed elution of hemagglutinating activity with both 0.01 M phosphate buffer pH 8 and 0.3 M phosphate buffer pH 6. In the light of the results of Reisner and Franklin(8) the above findings are compatible with the interpretation that 2-ME sensitivity was due to the presence of 19S antibody.

Several sera were chromatographed on Sephadex G-200. All samples containing 2-ME sensitive antibody exhibited hemagglutinating activity in that portion of the eluant appearing immediately after the exclusion volume. Those samples which in addition contained 2-ME insensitive antibody showed hemagglutinating activity also over a series of 4-5 fractions, which appeared shortly after the heavier antibody. A serum sample containing only 2-ME sensitive antibody and one containing both 2-ME sensitive and insentitive antibody were subjected to 105,000 *g* for 18 hours in sucrose gradients in a preparative ultracentrifuge. These results were in agreement with the chromatographic data.

Discussion. Several studies have been reported in which the type of antibody being stimulated by endotoxin has been characterized. Bauer and Stavitsky(9) reported that a *Salmonella* bacterial vaccine resulted in early appearance of a macroglobulin antidiphtheria toxin which lost hemagglutinating activity upon storage at +5°C or —20°C. Antitoxin from rabbits which had not been primed with *Salmonella* did not show this property. Our findings with sera that had been frozen and thawed confirm the instability of the antibody appearing early in another endotoxin stimulated species; but, in contrast, establish a similar instability for 2-ME sensitive antibody appearing in mice after treatment with antigen alone as well as antigen plus FUDR. In addition, Langevoort *et al*(10) and Rosenblatt and Johnson (11) have shown that the early antibody stimulated by endotoxin is of the 19S variety. A prolongation by endotoxin of synthesis of 19S antibody in several guinea pigs has also been reported by Uhr and Finkelstein(12).

The data recorded herein extend these results to another species, Balb and C58 mice, and document clearly the fact that 2-ME insensitive as well as 2-ME sensitive antibody is elevated by endotoxin. Reports by Mellors and Korngold(13) indicate that 7S and 19S gamma globulin are formed in the same cell type. No definitive evidence is available as to whether or not the same cell can make both types, but in any case the effect of endotoxin is probably manifested on total antibody response. The adjuvant action of FUDR appears to be similarly expressed on total antibody response.

Summary. Mice stimulated with human gamma globulin formed antibody which was mercaptoethanol sensitive and assumed to be of the 19S variety. Incorporation of bacterial endotoxin with the human gamma globulin stimulated antibody to appear earlier and to higher levels. This increased antibody response was due both to production of mercaptoethanol sensitive antibody and mercaptoethanol insensitive antibody. Injection of 5-fluoro-2-deoxyuridine one hour before the human gamma globulin also resulted in early

and enhanced levels of mercaptoethanol sensitive antibody, with an increase in the level of low molecular weight antibody appearing later. Similarly, C58 mice receiving endotoxin and BGG formed antibody earlier and to greater levels than did those receiving BGG alone. The increased antibody production in this strain also was due to both mercaptoethanol sensitive and insensitive antibody.

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Coenzyme A-Induced Inhibition of Conversion of Palmitic Acid to Ketone Bodies in Rat Liver Homogenates.* (29136)

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The oxidation of fatty acids to ketone bodies by the liver has long been recognized and the early literature on this phase of fat metabolism has been reviewed(1). A study of factors which affect the rate of ketone body formation from fatty acids in rat liver preparations has been undertaken in this laboratory. During this study the effect of added cofactors on the oxidation of short-chain and long-chain fatty acids to ketone bodies by a liver homogenate system was measured. It was observed that the addition of ATP, cytochrome C and coenzyme A together decreased the conversion of added long-chain fatty acids to ketone bodies. The individual cofactor responsible for this reduction in ketogenesis was then determined and the specificity of this inhibition was further studied. These observations are presented here.

Materials and methods. Male, Wistar rats, 3-5 months old, fed Purina Laboratory Chow *ad libitum* were decapitated after which the livers were rapidly removed and placed in ice-cold 0.9% NaCl. Liver homogenates, 12.5%, in Krebs-Ringer phosphate medium without calcium were prepared with a Potter-Elvehjem, all-glass homogenizer. Tissue homogenates were kept at ice bath temperature throughout their preparation until incubation in beakers in a Dubnoff shaker with a gas phase of air. Substrates and cofactors were added just prior to incubation. Caprylate solutions were adjusted to pH 7.4 with KOH before addition. Palmitate solutions were prepared by an alternate procedure(2) due to the more limited solubility of long-chain fatty acids. Coenzyme A was obtained from Pabst Laboratories. Similar results were obtained with coenzyme A from Sigma Chemical Co. At the end of incubation, proteins were precipitated by treating the homogenates with $CuSO_4$ and $Ca(OH)_2$(3) and filtered. Total ketone bodies in the filtrates were then determined(4). Endogenous ketogenesis, both

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