similar results were obtained and the myeloma proteins absorbed a sizeable amount of the total antibody. This interpretation was confirmed by the reaction obtained between normal gamma globulin and myeloma X antiserum. Through the medium of absorption of gamma globulin antiserum with purified myeloma proteins, a method was obtained for dividing the normal gamma globulin antiserum into two portions, a myeloma related portion and a non-myeloma related portion. Studies are under way in which these two antisera are being used. Preliminary observations have indicated that the increased gamma globulin found in the serum of patients with liver disease is of the myeloma related type. Further subdivision of the gamma globulin antiserum was possible by absorption with different myeloma proteins.

Since the myeloma proteins are found in the serum in huge concentrations in a disease characterized by great proliferation of plasma cells, it would appear that these cells are involved in the production of these proteins. Therefore, the results obtained showing a relationship between myeloma proteins and a portion of normal gamma globulin adds further evidence for a role by plasma cells in the production of at least a component of normal gamma globulin.

Summary. 1. Antisera against two preparations of normal gamma globulin reacted quantitatively with the special myeloma protein found in the serum of 4 patients with multiple myeloma. 2. The myeloma protein from the serum of a fifth patient showing the most rapid electrophoretic mobility failed to react with gamma globulin antisera. 3. Absorption of gamma globulin antiserum with purified myeloma proteins removed only a portion of the total antiserum, thus dividing the antibody into fractions. 4. Antiserum prepared against a highly purified preparation of a gamma$_2$ myeloma protein gave a precipitin reaction with normal gamma globulin.

The authors are indebted to Dr. Mary L. Petermann for furnishing two of the myeloma sera in addition to a purified preparation of myeloma protein and to Dr. Merrill W. Chase for furnishing adjuvants for immunization.

Received December 1, 1950. P.S.E.B.M., 1951, v76.

Adsorption of Bovine Alkaline Phosphatase on Diatomaceous Silica. (18433)

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(Introduced by G. W. Irving, Jr.)
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Recent reports have described the adsorption of enzyme-carrying cell particles(1,2) and viruses(3,4) on various types of Celite (diatomaceous silica) from salt-containing solutions. Similarly, when salts are present, a partially purified alkaline phosphatase of cow's milk(5) is strongly adsorbed on the Celite Filter-Cel. The adsorption affords some increase in the purification of the enzyme, since the non-phosphatase protein is less strongly adsorbed. The results, however, are of more interest in explaining the adsorption of certain complex proteins on Celite. A comparison of adsorption on various Celites suggested that the presence of clay in its natural state was necessary for adsorption. The influence of various physical and chemical factors on the adsorption was also investigated.

Materials and methods. The preparation

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of the alkaline phosphatase of cow's milk has been described(5). Since solutions of the phosphatase were somewhat unstable, the preparation was dried from the frozen state. The assays were performed in ethanolamine buffer as described(5). Enzyme activities are expressed as colorimeter readings, a reading of 1 being taken as 1 unit. The activity of the dry preparation was 225 units/mg. This preparation,† which is strongly adsorbed on Filter-Cel, was used in all the experiments. The adsorptions were performed in 0.1 M buffers: collidine, pH 7.06; tris-(hydroxymethyl)-aminomethane, pH 8.06; and ethanolamine, pH 9.65. Salts, principally NaCl and Na_2SO_4, and the dry milk phosphatase were added to the buffers. Filter-Cel was added to 10.0 cc of the enzyme-salt-buffer mixture. After stirring for 2 minutes, which was adequate for maximum adsorption, the mixture was centrifuged, and the supernatant solution was decanted and assayed for enzyme activity, together with the original enzyme-salt-buffer solution. In the elution experiments, 10.0 cc of eluent were added to the sedimented adsorbent, stirred for 2 minutes, and centrifuged, and the supernatant solution was assayed for activity.

Results. The effect of varying the amount of Filter-Cel with several concentrations of the milk phosphatase in 1.5 M NaCl, pH 8.0, at 23°, is shown in Fig. 1. The increase in adsorption of the phosphatase on dilution, with small amounts of Filter-Cel, as well as the shape of the curves, is characteristic of most adsorptions. The limit of adsorption, however, of about 80% for each dilution of

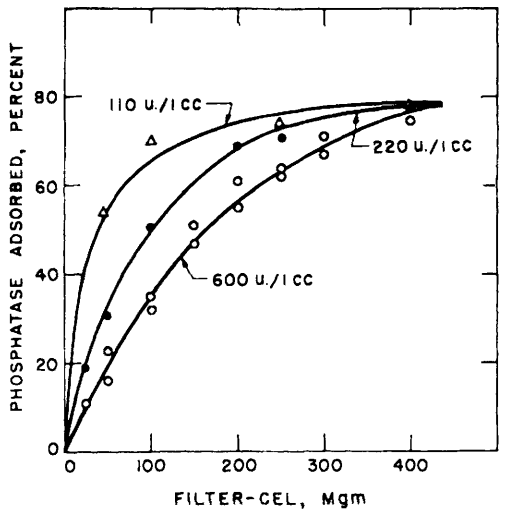

FIG. 1.
Adsorption of milk phosphatase on different amounts of Filter-Cel with several concentrations of the enzyme from 1.5 M NaCl, pH 8.0, at 23°.

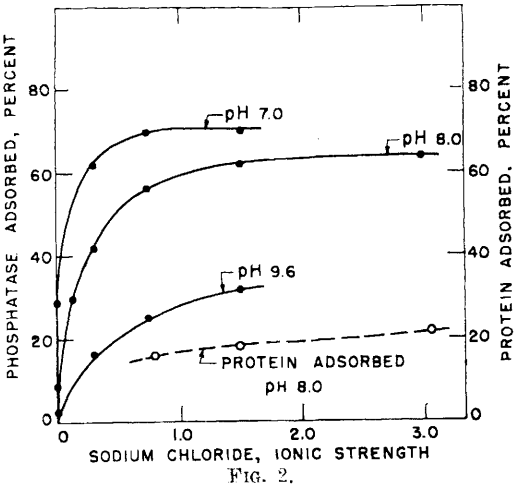

FIG. 2.
Effect of salt concentration and pH on adsorption of milk phosphatase on 250 mg Filter-Cel.

phosphatase is unusual and suggests that an unadsorbed phosphatase is present to the extent of 20%.

The influence of NaCl concentration and pH on the adsorption of the milk phosphatase on 250 mg of Filter-Cel is shown in Fig. 2. Although the results at each pH were obtained with a different buffer, the regular variation with pH suggests there were no specific effects attributable to the buffer compound. The percentage of the total protein

† Solutions of this phosphatase preparation are opalescent. Further purification has given clear solutions that are poorly adsorbed on Filter-Cel. These and other results suggest that the phosphatase adsorbed on Filter-Cel is a complex, perhaps lipid-containing. In the course of further purification in which treatment with lipase is employed, the complex is dissociated to give a phosphatase not adsorbed on Filter-Cel. The small amount of phosphatase not adsorbed on Filter-Cel from the preparation used in the present experiments may indicate the presence of the dissociated phosphatase. The mere presence of non-phosphatase protein would by interference be more likely to reduce rather than to increase adsorption of phosphatase.

adsorbed with several concentrations of NaCl at pH 8.0 is shown in Fig. 2 also. It is evident that if the enzyme could be totally eluted from the Filter-Cel, 3-fold purification of the phosphatase could be obtained in one treatment with Filter-Cel. The phosphatase concentration in these experiments was 600 u/cc.

Although the shape of each adsorption curve suggests an equilibrium between adsorbent and enzyme, elution of the enzyme activity never exceeded about 60%, with a consequent increase in purity of only 1.5- to 2-fold. When adsorbed on Filter-Cel from salt-containing buffers, none of the phosphatase could be eluted at the same pH even with salt-free buffer. However, the enzyme could be partly eluted by increasing the pH, for example, enzyme (600 u/cc) adsorbed on 250 mg of Filter-Cel at pH 8.0 from 1.5 M NaCl could be eluted to the extent of 37% with salt-free ethanolamine buffer at pH 9.6. The recovery was related to the amount of adsorbent; 17.5, 65 and 67%, respectively, were recovered from 500, 100 and 50 mg of Filter-Cel. Calculations showed that the non-recovered enzyme was proportional to the amount of Filter-Cel, namely, about 65 u/100 mg. This suggests that there is a firmly held portion of enzyme that cannot be eluted and a portion of enzyme adsorbed thereon or less reactive sites that can be eluted. The amount that can be eluted is increased when the ratio of enzyme to adsorbent is increased. This was also evident when 250 mg of Filter-Cel on which phosphatase had been adsorbed from 0.8 M Na_2SO_4, pH 8.0, and then washed with ethanolamine buffer, was used as adsorbent. Only 36% of phosphatase was adsorbed in this case, but 82.4% of it was eluted with ethanolamine buffer.

The recovery of phosphatase was greater when Na_2SO_4 was used instead of NaCl at the same ionic strength. With 250 mg of Filter-Cel, the recovery of enzyme adsorbed from Na_2SO_4 was 50% as compared with 37% from NaCl. Tests in which Na caprylate, Na citrate, NaK tartrate or K thiocyanate replaced the Na_2SO_4 led to no improvement in the elution. Adsorption increased with rise in temperature (5° to 37°), but variation of this factor in the elution offered no advantage. When tested in 250-mg portions at pH 8.0 with Na_2SO_4 present, the following adsorbents adsorbed the phosphatase only slightly (0 to 14%); Alumina (80-200 mesh), talc, α-cellulose, filter paper, Norite, calcium phosphate, and Lloyd's reagent. Bentonite gave a strong adsorption (72% from 0.4 M Na_2SO_4). The phosphatase was not eluted with ethanolamine buffer, but it was eluted with 0.1 M collidine to the extent of 53%. Interesting results were obtained with Celites other than Filter-Cel. At pH 8.0, with 1.5 M NaCl and 250 mg of the Celite, Standard Super-Cel adsorbed 12% of the phosphatase, and Hyflo Super-Cel only 3% as compared with 62% for Filter-Cel. Data supplied by the manufacturer show that Filter-Cel, and Standard and Hyflo Super-Cels have very much the same particle size. Particle size, therefore, cannot account for the differences in adsorption noted. These Celites, however, differ in other respects. Filter-Cel is a natural product, whereas Standard Super-Cel is calcined, and Hyflo Super-Cel is calcined after a flux has been added. It has been reported(7) that calcination converts the hydrated colloidal clay, present in Celite to the extent of about 4% (as Al_2O_3), to the less adsorbent anhydrous aluminum silicate. This suggests that the adsorption of the milk phosphatase shown by Filter-Cel may be due largely to its content of clay.

Discussion. The effect of increasing ionic strength on the absorption of milk phosphatase parallels the results of Shepard and Tiselius(8) for the adsorption of serum albumin on silica gel. The studies are alike also in showing a decrease in adsorption at higher pH values.

Irreversibility of adsorption, noted in the

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experiments reported here, has also been noted by Mitchell *et al.*(9) in studies of amylase, adenosine deaminase and "adenylic acid phosphatase" in Taka-diastase on their filter paper chromatopile. In an initial run with a $(NH_4)_2SO_4$ gradient at pH 6.5, the enzymes were separated, the "phosphatase" being least adsorbed(9). In second runs with samples from activity peaks, however, the enzyme failed to move. Presumably a strongly adsorbing component of the Taka-diastase was acting as a displacing agent in the first run. The enzymes could be made to move in the second runs by raising the pH to higher levels. Runs at a pH of 6.5 and constant $(NH_4)_2SO_4$ concentrations gave a wide spread of the enzyme activity, with a frontal peak in most cases. Such results would follow from the present observations on two types of adsorption, that firmly held and that which can be eluted, the latter perhaps being held on the former.

The adsorption of the milk phosphatase on various Celites is roughly paralleled by the adsorption of certain viruses on Celites, suggesting that with viruses also the clay in the Celites might be the active adsorbent. The pneumonia virus of mice, for example, is strongly adsorbed at pH 6.8 from 0.15 M NaCl on Filter-Cel, Celite no. 505, and Super-Cel, less strongly on Hyflo Super-Cel and Celite no. 503(4). Here also elutions were successful only at high pH values, pH 9 and above.

Summary. The adsorption of a preparation of bovine alkaline phosphatase on Filter-Cel was investigated in respect to amount of adsorbent, concentration and kind of salt, pH and temperature. Calcined Celites were poor adsorbents for the phosphatase.

Received December 6, 1950. P.S.E.B.M., 1951, v76.

Effects of Vitamin B_{12} on Diethylstilbestrol and Thyroprotein Action in Male Rats.*† (18434)

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Recently vit. B_{12} has been demonstrated to counteract the inhibition of growth induced by feeding large doses of thyroid-active substances(1-4) or thiouracil(5) to young rats. It was considered possible that vit. B_{12} might likewise counteract the inhibition of growth which has been observed in young rats treated with diethylstilbestrol(6). It was also of interest to determine whether the effectiveness of vit. B_{12} in these respects could be explained by an increase in food consumption and/or an increase in the catabolism or rate of excretion of the hormones administered.

Methods. Immature male albino rats (Carworth strain) were divided into 10 uniform groups of 10 each, and were fed the following basal ration for 20 days: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; whole milk powder, 20%; alfalfa leaf meal, 6%; brewers' yeast, 3%; and table salt, 1%. Diethylstilbestrol‡ or thyroprotein‡ were mixed into the ration in amounts of 0.10 and 0.16 per cent respectively. Vit.

* Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1184.

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