mal rabbits of large doses of cortisone acetate under experimental conditions closely resembling those employed in the present study also produces a significant decrease in the exchangeable potassium content(8). The results of the present study suggest that one of the effects of digitoxin, administered in the dosages and in the manner employed for these experiments, is to deplete the body store of potassium. This depletion is thought to be the result of an extracardiac effect of digitoxin.

Summary. 1. The daily intravenous administration of 0.1 mg of digitoxin per kg of body weight to normal rabbits resulted, within 7 days, in an increase in body weight and a reduction in the serum potassium concentration and the exchangeable potassium content. These changes in potassium metabolism were corrected within one week by discontinuing the glycoside. 2. The intravenous administration of a single large dose of digitoxin (0.2 mg per kg) to normal rabbits resulted within 24 hours in a decrease in the mean potassium content of all the tissues and organs. Statistically significant decreases in potassium content were noted in the abdominal and diaphragmatic muscles and in the subcutaneous connective tissue. The results suggest that one of the effects of the administration of digitoxin in these dosages is to deplete the body store of potassium.

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A Practical Method for Removal of Anticomplementary Properties from Human Serum. (22026)

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Methods for the removal of anticomplementary (AC) qualities from sera(1-11) are generally too complex or inconvenient for frequent use in complement-fixation (CF) tests. Since Coons and his colleagues have found that absorption of fluorochrome-labeled antibody with tissue powder reduces or removes nonspecific staining activity(12,13) the effect of tissue powder on the AC properties of human sera was investigated. Sera were freed of AC activity by the relatively simple procedure of treatment with mouse liver powder.

Materials and methods. To make tissue powder, livers from normal adult white mice (Albany strain) were cut into 10- x 10-mm pieces after the removal of fat and connective tissue, and homogenized in a Waring blendor with distilled water (30 ml/100 g). The suspension was centrifuged and the supernatant removed. The sediment was washed 3 times in distilled water, then in 0.85% NaCl solution, and mixed thoroughly with 4 volumes of acetone. The acetone was removed after centrifugation and one volume of acetone was added to the sediment. The acetone was

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evaporated overnight at room temperature in Petri plates. Some samples were further dried in a vacuum desiccator over $CaCl_2$. *Virus and rickettsial antigens.* Virus antigens were prepared in this laboratory. St. Louis encephalitis antigen was a suspension of infected mouse brain(14) inactivated by ultraviolet radiation. The rest were made from tissue or fluids of infected embryonated hens' eggs. Eastern and Western equine encephalomyelitis antigens were extracts of embryo and chorioallantoic membrane suspensions in allantoic fluid also inactivated by ultraviolet radiation. Herpes simplex antigen was an extract of frozen and thawed chorioallantoic membranes. Mumps antigen was dialyzed allantoic fluid. Preparation of the soluble influenza antigens was based on Hoyle's method(15). Methods for production of psittacosis(16) and lymphocytic choriomeningitis(17) antigens have been described. All the virus antigens were stored in the dry state. Rickettsial antigens were Lederle Laboratory products. *Treatment of sera.* Serum was added to the liver powder in the proportion of 200 mg of powder/ml (50 mg/1 ml of 1:4 dilution) and shaken for one hour in a wrist-action machine.‡ The mixture was centrifuged for 30 minutes at 2000-2500 r.p.m. and the supernatant removed from the sedimented liver powder with a fine Pasteur pipette, then inactivated for 30 minutes at 60°C. *Complement-fixation tests.* Treated and untreated sera were appropriately diluted and examined in a quantitative CF test(18) with one, two, and three 50% units of complement in the absence of antigen to determine anticomplementary titer. Serial 2-fold dilutions of serum, beginning with 1:4, were tested with various antigens and three 50% units of complement. Fixation for 18-24 hours at 3°-6°C was followed by incubation for 30 minutes at 37°C with sensitized cells. Antigen controls, complement controls, with one, two, and three 50% units, and a control for sensitized cells were included; and tests with treated and untreated "positive" and "negative" control sera were included with each group of anticomplementary sera examined. Titers were expressed as the number of 50% complement units fixed by undiluted serum. Sera were considered AC if two 50% units of complement induced less than 90% hemolysis in a 1:4 serum dilution in the absence of antigen. The calculated minimum number of 50% complement units fixed by the least AC sera was 5.7 according to this criterion. Many of the sera selected for study were more strongly AC.

‡ Burrell Technical Supply Co., Pittsburgh, Pa.

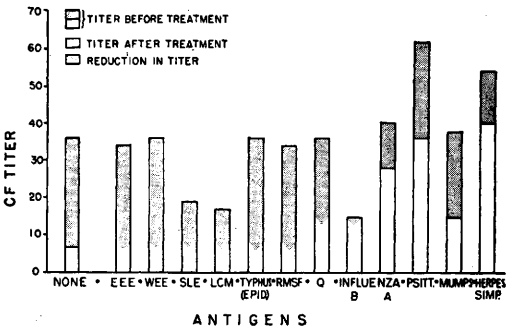

FIG. 1. Effect of liver powder treatment on strongly AC Pool I (20 sera) in CF tests with 12 different antigens. EEE, WEE, SLE = Eastern, Western, and St. Louis encephalitis; LCM = lymphocytic choriomeningitis; RMSF = Rocky Mountain spotted fever.

Results. The objects of the experiments were: To determine the extent to which liver powder removed or inhibited AC activity; to find out if specific antibody titers were altered; and to ascertain whether nonspecific CF properties were introduced by liver powder treatment. Accordingly, both AC sera and sera free of AC activity were examined in CF tests in the absence of antigen, giving the so-called "serum control titer"; in the presence of specific virus or rickettsial antigens; and in the presence of "control" antigens prepared from uninfected tissues or fluids.

Anticomplementary sera. To determine whether liver powder treatment would reduce or remove AC activity, AC sera were pooled and tested prior to and after treatment. Fig. 1 shows the results with one strongly AC pool examined with 12 virus or rickettsial antigens. The untreated serum control fixed thirty-six 50% units of complement, the treated control 6.8, a reduction of 29 units. In all cases, "control" antigens fixed <8 units of complement after treatment. The titers with 6 of the specific antigens were also reduced to <8

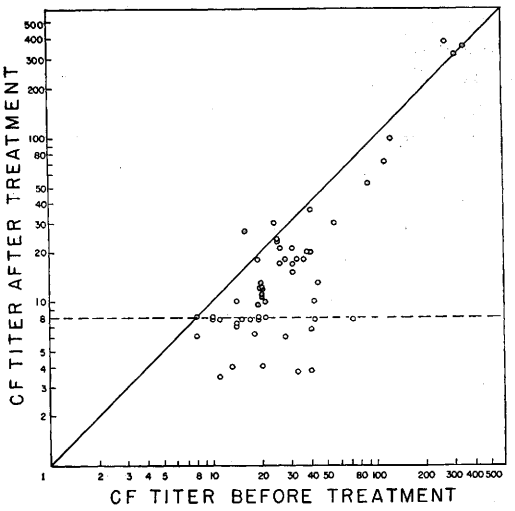

FIG. 2. Effect of treatment on 32 AC sera in 56 tests with 7 different virus or rickettsial antigens.

after treatment, a level considered nonreacting in the test employed. With 4 other antigens, however, namely herpes simplex, mumps, psittacosis, and influenza Type A, some activity remained after liver powder treatment. The reduction corresponded to that obtained in the absence of antigen. It was reasonable to assume, therefore, that the pool contained antibodies to these viruses, and that the procedure did not destroy or denature the antibody. Confirmatory results were obtained with a second moderately AC pool.

In the next experiment, untreated and treated portions of 32 individual AC sera were similarly examined. Liver powder treatment reduced the AC activity of all but one serum, and 22 of the 32 were freed of AC properties, *i.e.*, they fixed less than 5.7 fifty percent units of complement in the absence of antigen. Five of the remaining 10 sera fixed <8 fifty percent units of complement, but 4 markedly AC sera were not affected as decidedly.

To estimate further the effect of treatment on AC activity on these 32 sera, 56 tests were done with different virus or rickettsial antigens and 50 tests with their corresponding uninfected tissue or fluid control antigens. Liver powder treatment decreased the amount of complement fixed with the virus or rickettsial antigen in all but 6 instances (Fig. 2). In 80% of the sera tested, this decrease closely approximated the reduction in AC activity. Table I gives the results obtained with 5 sera from this group tested with various antigens.

Sera free of anticomplementary activity. More conclusive evidence regarding the effect of liver powder treatment on antibody was

TABLE I. Representative Changes in CF Titers of AC Sera Treated with Liver Powder.

Serum No.	Test	Titers: Pre-treatment* A	Titers: Post-treatment* B	Titer differences A–B†
2072	No antigen (AC activity)‡	26	12	14
	With "control" antigen§	20	14	6
	With influenza Type A antigen	36	18	18
1159	No antigen (AC activity)	18	5.3	12.7
	With "control" antigen	20	<8	>12
	With psittacosis antigen	56	30	26
2462	No antigen (AC activity)	16	5.6	10.4
	With Q fever antigen	19	<8	>11
2205	No antigen (AC activity)	19	6.4	12.6
	With "control" antigen	22	9	13
	With influenza Type A antigen	33	18	15
	Idem B	31	17	14
	" C	28	18	10
2091	No antigen (AC activity)	31	22	9
	With "control" antigen	26	21	5
	With mumps antigen	42	30	12

* Pre- or post-treatment with liver powder.
† A–B = Effect of liver powder in decreasing titer.
‡ Determination of AC activity by test in absence of antigen ("serum control").
§ Test in presence of uninfected tissue or fluid antigen corresponding to virus or rickettsial antigen.

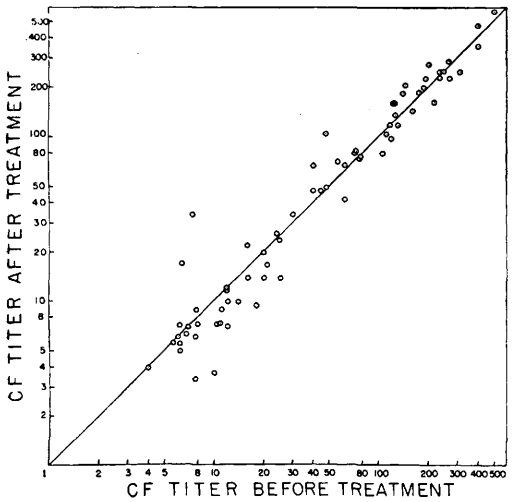

FIG. 3. Effect of treatment on 41 sera free of AC activity in 70 tests with virus or rickettsial antigens.

obtained from studies of sera that had no AC activity. Forty-one sera with titers ranging from <8 to 497 were investigated in 70 tests with virus or rickettsial antigens. Titers of these sera were virtually unaltered by liver powder treatment (Fig. 3). Only 13 (19%) pre- and post-treatment titers differed by more than 25%.

Discussion. Removal of anticomplementary activity without destruction or denaturation of specific complement-fixing antibodies has been a problem from the early days of the CF test. Sachs'(1) method of treatment with diluted HCl depends upon formation of a precipitate that carries down most or all of the AC activity. Maltaner and Maltaner(2,3) modified Sachs' technic. They adjusted the pH of sera to various levels, and concluded that the amount of AC activity removed was proportional to the euglobulin precipitated. For optimum results, it was necessary to adjust the relative concentrations of reagents for each serum. The method was therefore not suitable for large-scale application. Davis *et al.*(4) showed that the AC properties of electrophoretically separated γ-globulin were neutralized by appropriate amounts of β-globulin or albumin. They also investigated high-speed centrifugation as a method for removing AC activity, but concluded that its usefulness is limited. Taran(6) and Maaløe(7), like Davis *et al.*, attempted to neutralize rather than to remove the substances responsible for AC activity. They adopted the direct method of adding complement to replace the amount bound nonspecifically by the AC substances. This method, however, has the disadvantage of decreasing or altering antibody titers; under these conditions, mixtures of complement that are apparently identical with respect to hemolytic potency may differ considerably in their ability to combine with antigen-antibody complex(19). Like the other methods, this technic has the limitation that the appropriate dose of reagent, in this case complement, must be determined for each AC serum.

The method described here is empirical. The effect of liver powder in removing or neutralizing AC properties was studied because tissue powder treatment prevents nonspecific fluorescence(12). The treatment was also highly effective in removing or reducing the AC properties of most of the sera studied, did not reduce specific CF antibody titer in most instances, nor introduce other nonspecific properties that interfere with the CF test to an important degree. Fractionation of the powder would be a promising approach toward explanation of the mechanism of action. Other tissue powders are also being examined for their ability to remove or reduce AC activity.

Preliminary studies suggest that the effect of liver powder on the AC properties of γ-globulin is worth investigation(20). Some results indicate that the method might find application in serologic tests for syphilis with cardiolipin antigen(20). The procedure lends itself well to relatively large-scale work because it is technically simple. In particular, the effective amount of liver powder does not need to be determined separately for each serum specimen. It seems worth while, therefore, to present our data for their possible practical value before an explanation is at hand, in the hope that these observations may prompt further studies of the reaction.

Summary. A simple method for the removal of anticomplementary properties from sera by treatment with acetone-extracted, dried normal mouse liver powder is described.

This material removes nonspecific properties, does not markedly reduce or increase antibody titers with sera giving specific reactions with virus and rickettsial antigens, and does not introduce nonspecific properties in nonreacting specimens.

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Inhibition of Development of Chick Embryo Liver by Aminoguanidine. (22027)

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Studies have been made on the nutrition, metabolism, and development of the chick embryo by injection of biologically active substances into the embryonated egg. Profound inhibition of growth with production of developmental abnormalities result from injection of certain substances(1-5), and Karnofsky(6) pointed out the value of this technic as a screening method for specific growth-inhibitors.

By extension of this technic to new classes of chemical compounds, we have observed a severe inhibition of the development of the chick embryo liver by aminoguanidine. This inhibition is rather specific and has no obvious effect on the other organs of the body under the conditions described.

* The authors gratefully acknowledge the technical assistance of Mr. Gene Conway and Mr. Merle Maxwell.

Methods. Aminoguanidine sulfate (AGS)† was dissolved in double distilled water to form

TABLE I. Effect of Aminoguanidine Sulfate on Mortality, Body and Liver Weight of Chick Embryo.*

Quantity injected (mg/egg)	No. of eggs	Embryos survived	Body wt (g)†	Liver wt (mg)†
H_2O	4	4	9.89 ± .89	173 ± 26
2	14	12	8.26 ± .96	139 ± 38
5	8	7	8.58 ± .52	66 ± 39
10	14	8	8.07 ± .69	74 ± 44
15	8	8	9.26 ± .71	65 ± 62
20	14	9	7.11 ± .79	45 ± 36‡
25	8	1	6.75 ± —	— —
40-60	9	0	— —	— —

* Yolk sac injections were made on 4-day-old embryos which were harvested 10 days later.

† Mean value ± avg dev.

‡ Six of the livers at this dosage each weighed less than 25 mg.

† Obtained from Distillation Products Industries, Eastman Organic Chemicals Department.