the rabbits treated with these two substances. The results of our work are interpreted as strongly suggestive that glucagon originates from the A cells. Quantitative determination of the glucagon content of pancreas treated with drugs toxic for the A cell together with detailed morphological study of the type of A cell lesion, will aid in the elucidation of this problem.

Summary. Pancreatic extracts of cobalt-treated guinea pigs with a 4+ lesion fail to produce a significant hyperglycemia when assayed in cats; however, if well-granulated A cells remained in amounts roughly estimated above 25% of the normal, the extracts from these pancreases invariably elicited a hyperglycemia indistinguishable from that of the normal. These results are interpreted as strongly suggestive that glucagon originates from the A cells.

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Effect of Splenectomy on Antibody Formation in Cortisone Treated Rats. (22340)

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The hemolysin formation in rats after a single intraperitoneal injection of sheep erythrocytes was the object of a previous study (1). It was suggested that at a large dose of the antigen (about 2 ml of a 10% suspension) a considerable part of the sheep cells enters the blood stream and is taken up by the spleen. The spleen may then be responsible for a large fraction of the circulating antibodies measured. However, at an antigen dose 30-40 times smaller, splenectomy preceding(2,3) or subsequent to (unpublished experiment) the intraperitoneal antigen injection did not appreciably alter the hemolysin response. In another series of experiments some factors which condition the antibody-reducing effect of cortisone were studied(4). It was shown that the hemolysin response after a large intraperitoneal dose of sheep cells was less affected by cortisone than that after a small one. One possible explanation involved the assumptions: 1) that the spleen produces a dominating part of the hemolysins with a large antigen dose but not with a small one, and 2) that splenic antibody production is more resistant to cortisone than is extrasplenic antibody formation. The effect of cortisone should then be less with a large than with a low antigen dose.

The following experiment was an attempt to verify the two assumptions.

Material and methods. Animals. White

TABLE I. Effect of 4 mg Dose of Cortisone on Hemolysin Response in Sham Operated and Splenectomized Rats. Animals were immunized by one intraperitoneal injection of sheep erythrocytes on day 0.

Group	Operation	Treatment	Initial avg wt, g	Hemolysin titer, mean ± stand. error of mean† Day +4	Day +5	Day +8
I	Sham	C* day −8 to +1	208	3.26 ± .52 (9)	6.81 ± .38 (9)	6.77 ± .55 (9)
II	"	No	204	5.19 ± .45 (8)	7.50 ± .29 (8)	6.41 ± .36 (8)
III	Splenectomy	C day −8 to +1	200	.00 (10)	.57 ± .37 (10)	1.40 ± .62 (9)
IV	"	C day −2 to +1	196	.07 ± .03 (9)	2.77 ± .47 (9)	3.94 ± .49 (9)
V	"	No	214	1.87 ± .49 (7)	5.57 ± .43 (7)	5.37 ± .48 (6)

* C = Cortisone. † Figures within parentheses are No. of animals.

male rats weighing 170-200 g were allotted at random to the experimental groups(1). Blood samples of 1 ml were taken from tails. *Splenectomy and sham operation.* All operations were performed under evipal sodium anesthesia on the 1st day after the antigen injection. After midline abdominal incision the spleen was exteriorized. In the sham operation, the spleen was replaced in the abdomen. When splenectomy was performed the spleen was removed after ligation of the pedicle. *Hormone.* Cortisone acetate* was given as daily intramuscular injections in a dose of 4 mg per 100 g body weight (designated "the 4 mg dose"). *Antigen.* Fresh sheep erythrocytes were washed 3 times with saline and diluted with saline to a 10% suspension. A small quantity of India ink (dilution in antigen 1:2000) was added. The rats were immunized by a single intraperitoneal injection of 1 ml of the antigen suspension per 100 grams initial body weight (Table I). At the end of the experiment all animals were autopsied to determine whether antigen had been deposited in the peritoneal cavity (traces of carbon). The day of antigen injection is called day 0, days before and days after are conventionally indicated by minus and plus respectively. *Titration of hemolysin.* Inactivated sera were titrated in 2-fold serial dilutions. Hemolysins were determined by a standard procedure(1) using 4 units of complement, incubation at 37°C for 30 minutes, and subsequent centrifugation. The degree of hemolysis was estimated by the aid of a hemolytic scale. The 50% end point was calculated by interpolation. The titers are expressed as the negative ^{2}logarithms of the serum dilution, taking dilution 1:6.25 as a provisional zero point (1:12.5 = 1; 1:25 = 2, etc.). All sera from one bleeding were titrated simultaneously.

Experimental. A scheme of the cortisone treatments and the operations is given in Table I. Two groups of 12 rats were given the 4 mg dose of cortisone for 10 days beginning 8 days before the antigen injection (I and III). One group of 9 rats received the same dose of cortisone for 4 days beginning 2 days before the antigen injection (IV). Another 2 groups of 9 rats had no treatment (II and V). One day after antigen injection, splenectomy was performed on the following groups: Group III, treated with cortisone for 10 days; Group IV, treated for 4 days, and Group V, non-treated. The remaining 2 groups (I and II) were sham operated. Five animals died during or soon after the operation. Another 5 animals died later, probably due to infections. In 3 of these the intraperitoneal deposition of the antigen could not be verified. The data of these rats have been excluded. The animals were bled on the 4th, 5th and 8th day after the antigen injection.

Results. Splenectomy produced a significant reduction of the hemolysin level on day +4 (Fig. 1 and Table I). This effect appeared to be less pronounced on day +5 and was no longer significant on day +8.

In sham operated animals the 10-day cortisone treatment produced a probably significant ($P<0.02$) titer reduction on day +4 (Table II). At the later bleedings this effect was no longer manifest. In splenectomized rats the same treatment had a significant effect on all bleeding days. On day +5 and +8 the 10-day cortisone treatment seemed to be much more effective in splenectomized

* Cortone, Merck and Co., furnished through kindness of Erik Lindblom and Co., Stockholm.

TABLE II. Mean Titer Difference between Non-Treated Groups (II and V) and the Respective Similarly Operated Cortisone-Treated Groups (See Table I).

Operation	Cortisone, No. of days	Titer reduction, mean ± S.E. of mean Day +4	Day +5	Day +8
Sham	10	1.93 ± .69*	.69 ± .49	−.36 ± .67
Splenectomy	10	≥1.87 †§	5.00 ± .57‡	3.97 ± .86‡
"	4	1.80 *§	2.80 ± .66‡	1.43 ± .72

* Significance level 5%. † Significance level 1%. ‡ Significance level .1%.
§ This significance was obtained by comparison of intra-group distributions of values above and below the general mean of the two groups, see Mainland(7).

animals than in those sham operated. To test the significance of this difference, an analysis of variance(5) was used with two bases of classification: splenectomy and cortisone treatment (10 days or none). The interaction term proved the difference significant at the 0.001 level.

In splenectomized animals cortisone treatment for 4 days produced a significant reduction of the hemolysin level on day +5 but not on day +8 (Table II).

Discussion. Splenectomy produced a significant reduction of the hemolysin level only on the 2 first bleeding days (Fig. 1). Thus the splenectomized animals seemed to have a slower rise of antibody but may actually have formed as much antibody as the controls. Taliaferro and Taliaferro(6) have reported similar observations in rabbit experiments where the antigen was given intravenously. These authors suggest "that the spleen in intact animals usually forms a large part of the antibody which accumulates to give the initial peak serum titer, and then abruptly ceases to form antibody." Such an early transient role of splenic hemolysin formation may also explain the present findings.

Administration of the 4 mg dose of cortisone for 10 days, begun 8 days before the antigen injection, was significantly more effective in splenectomized than in sham operated animals on day +5 and +8. In fact cortisone treatment for 4 days, started 2 days before the antigen injection, was enough to produce a significant reduction of the antibody level on day +5. In non-splenectomized animals this short period of hormone administration has failed to significantly affect the hemolysin response to this antigen dose (about 2 ml of a 10% suspension) but has repeatedly suppressed the antibody level on day +5 at a 30 times smaller sheep cell dose (4).

The present results indicate that splenectomy has lowered the resistance to cortisone of the hemolysin formation after a large intraperitoneal antigen dose. The mechanism of this effect is not clear. The result of splenectomy in animals not treated with cortisone (c.p. day +8, Fig. 1) does not lend support to the hypothesis that most of the hemolysins, produced after a large antigen dose, emanate from the spleen. Consequently the present data do not indicate whether or not the splenic antibody formation is more resistant to cortisone than the extra-splenic. The experiment has not clarified the cause of the

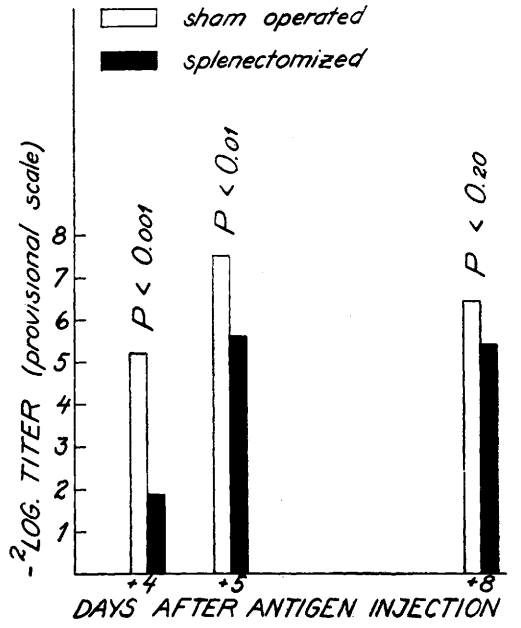

FIG. 1. Effect of splenectomy on average hemolysin response after a single intraperitoneal inj. of sheep erythrocytes. P values indicate level of significance of mean difference.

increased resistance of hemolysin production after a large intraperitoneal sheep cell dose.

Summary. 1. Splenectomy, performed one day after an intraperitoneal injection of a large dose of sheep erythrocytes, reduced the initial part of the hemolysin response, but did not significantly affect a later part. 2. The antibody-reducing effect of cortisone was significantly greater in splenectomized than in sham operated rats.

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Simplified Method for Calculating Flow, Mean Circulation Time and Downslope from Indicator-Dilution Curves. (22341)

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The use of indicator-dilution curves to measure cardiac output and "central blood volume" is widespread and of proved value (1-4). When the curves are markedly prolonged, as in heart failure, the calculations as ordinarily performed are time consuming because of the necessary integrations of the curves. Two methods of integration of the curves are in general use. The first consists of arithmetic summation of all concentrations each second from appearance time to some time later—usually through one or two cycles of semi-logarithmic paper. The second method involves replotting the curves (after extrapolation on semi-logarithmic paper) on linear paper and integrating planimetrically. The calculation of mean circulation time is done by finding the center of gravity of the linearly plotted curve on cardboard(2), or else by summing arithmetically all the products of time and concentration from the semilogarithmic plots and dividing by the arithmetic or planimetric integration. Lewis(5), described an ingenious method of calculating the cardiac output from indicator-dilution data which eliminated the necessity for plotting the curves. Though generally useful, this method does not permit calculation of mean circulation time. In addition errors in samples which might become apparent from plots of the data could be missed more easily.

This paper proposes a method for accurately obtaining the integral, slope and mean circulation time of an indicator-dilution curve in a short time, with relatively simple calculations. The method involves a combination of arithmetic integration (summing of concentration) of a small portion of the curve, and mathematical integration of the remainder.

Method. Semilogarithmically plotted indicator-dilution curves generally can be divided into two portions. The first is the rapidly rising upslope and the curved portion of the downslope; the second is the linear portion of the downslope, extrapolated toward infinite time. It becomes apparent that the integral of the total curve is the sum of 2 areas—the area from appearance time to beginning of linearity of the downslope, and the area from the beginning of the downslope to infinite time (Fig. 1). The area of the first portion can easily be obtained by summation. Because of the steep rise and relative sharp-

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