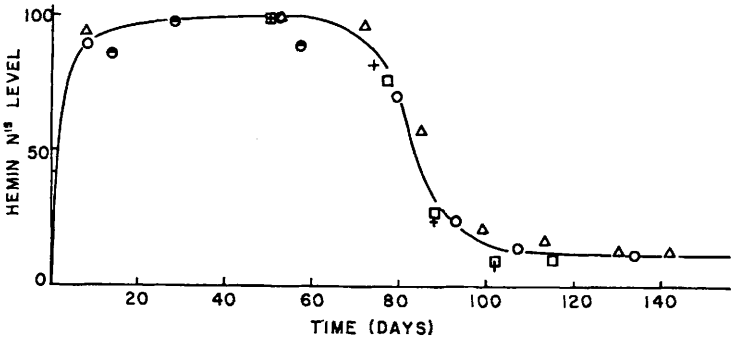

FIG. 1. Composite curve for 5 animals derived from corrected hemin N^{15} excess ratios expressed as per cent of plateau values.

mally labeled (about 50 days). Further, it was apparent that repeated sampling of blood, the volume of each sample being approximately 10% of the circulating blood volume, was in itself decreasing the hemin isotope level. Accordingly, two corrections were applied to the data to make them more comparable and to provide a mean curve from which the half-life of the red cell could be calculated. First each value was multiplied by a factor derived from the sample volume and the cat's blood volume, itself corrected for dilution by previous sampling. Second, observed N^{15} excess ratios, corrected for sampling, were expressed as percentages of the plateau N^{15} level for that animal. The corrected data are presented in Fig. 1.

Using the method of calculation presented by Shemin and Rittenberg(9), the mean life span, corrected for the initial labeling period, was determined to be 77 days, while half of the cells had a life span between 72½ and 81½ days.

Summary. The mean life span of the erythrocyte in the cat as determined by labeling heme with N^{15} is 77 days.

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Passive Sensitization of Human Skin by Sera of Guinea Pigs Anaphylactically Sensitized to Ovalbumin. (18738)

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Previous studies have shown that immediate urticarial reactions of the Prausnitz-Kustner type may be produced in human skin after passive sensitization with sera from rabbits sensitized with ovalbumin and with sera from guinea pigs sensitized to pollens of grass or ragweed(1,2). The present study concerns the activity in passively sensitizing human skin of sera from guinea pigs sensitized with crystalline ovalbumin, and the relationship of that activity to the capacity of the same antisera to produce passive anaphylactic sensitivity in the guinea pig.

Samples of sera obtained from guinea pigs sensitized by various methods at the Allergen Research Division of the Department of Agriculture were tested in the passive sensitization of human skin at Roosevelt Hospital. Other

1. Sherman, W. B., Menzel, A. E. O., Seebohm, P. M., *J. Exp. Med.*, 1950, v92, 191.

2. Sherman, W. B., Stull, A., and Hampton, S. F., *J. Immunol.*, 1939, v36, 447.

portions of the same antisera were tested in the passive anaphylaxis of guinea pigs at the Allergen Research Division.

Methods. To determine activity in passive anaphylaxis, serial dilutions of each serum (differing by a factor of 1.5) were injected intraperitoneally into normal guinea pigs weighing 280-310 g. These animals were challenged 2 days later with intravenous injections of 1 mg of ovalbumin. Assays were considered satisfactory if 2 of the serial dilutions produced nonfatal reactions and the 2 succeeding lower dilutions produced fatal anaphylaxis. The anaphylactic titer was expressed in terms of the number of fatal sensitizing doses per ml of serum. Skin-sensitizing activity was determined by injecting 0.1 ml of serum intracutaneously on the back or upper arm of a normal human test subject, shown by previous skin tests not to be allergic to ovalbumin or guinea pig serum. Twenty-four to 48 hours later 0.05 mg of ovalbumin, dissolved in 0.025 ml of saline, was injected intracutaneously into the same site. All sera were tested on two different volunteers. Reactions were observed after 15 minutes. Sites showing positive reactions were retested in serial dilutions to measure the skin-sensitizing antibody, the titer being the highest dilution producing a distinct reaction.

Sera from 8 guinea pigs sensitized to ovalbumin, which showed anaphylactic titers of 13-40 fatal sensitizing doses per ml, all failed to passively sensitize human skin. These animals were sensitized by single subcutaneous injection of 0.002-0.3 mg of alum-precipitated ovalbumin. They were bled 3-32 weeks after the sensitizing injection. Sera from 2 guinea pigs sensitized with 3 such subcutaneous injections (0.3 mg each) and bled 4 weeks after the last dose likewise failed to sensitize human skin, but showed anaphylactic titers of 13 and 20. Two guinea pigs were sensitized by intraperitoneal injections of ovalbumin solution in saline. One was bled 4 weeks and the other 32 weeks later. Anaphylactic titer of the first serum was less than 1.7; the titer of the second serum was 5. These antisera showed no skin-sensitizing activity.

Another series of 9 ovalbumin antisera was prepared from 7 guinea pigs that had survived anaphylactic shock, either once or twice. (Table I). These animals had been sensitized 6 to 8 weeks previously with a single subcutaneous injection of 0.02-0.3 mg of alum-precipitated ovalbumin. All 7 guinea pigs were bled 6 to 13 days after surviving a moderate or severe shock induced by intravenous injection of 0.077-0.59 mg of ovalbumin. Two of these guinea pigs survived a second intravenous challenging injection of 1 mg of ovalbumin 18 days after the first and were again bled 11 days after the second shock.

TABLE I. Skin-sensitizing and Anaphylactic Activity of Sera of Guinea Pigs Surviving Challenging Doses of Ovalbumin.

Guinea pig No.	Sensitizing dose,* mg	Interval before challenge	Challenging dose,† mg	Interval before bleeding, days	Anaphylactic titre	Skin sensitizing titre
160	.02	6 wk	.077	6	40	0
195	.02	6 "	.260	6	100	1-100
180	.3	6 "	.173	11	133	0
181	.3	6 "	.115	11	100	0
706	.3	8 "	.39	13	130	1-10
	s. c.‡	18 days	1.0	11	200	1-10
707	.3	8 "	.59	13	400	0
711	.3	8 "	.26	13	200	1-100
	s. c.	18 days	1.0	11	130	1-10

* Alum precipitated ovalbumin subcut.
† Intrav., in saline sol.
‡ s. c. = second challenge.

All 9 antisera induced passive anaphylactic shock; the titers ranged from 40 to 400 doses per ml. Antisera from 3 of the 7 guinea pigs passively sensitized human skin in dilutions of 1:10 to 1:100, the other 4 did not, even when undiluted. There was no significant difference either in anaphylactic titer or skin-sensitizing capacity between the sera prepared after the first, and after the second challenging injection. No relationship between anaphylactic titer and skin-sensitizing capacity was apparent.

Thus, sera from some guinea pigs sensitized with ovalbumin produced passive sensitization of human skin, but sera from others similarly treated were inactive in this respect. All of the antisera showing skin-sensitizing activity were from guinea pigs which had survived anaphylactic shock following challenging in-

jections. However, no antisera were obtained from these pigs before the shock dose. Hence, the effect of the challenging injection on the formation of this type of antibody could not be assessed.

The skin-sensitizing activity of the sera bore no relation to their potency in passive sensitization of guinea pigs. This observation permits the speculation that skin-sensitization and anaphylaxis are mediated by different antibodies, the skin-sensitizing antibody being formed by only a fraction of the guinea pigs in response to a type of stimulation which consistently produced anaphylactic antibody.

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Certain Relationships Between Pteroylglutamic Acid, Citrovorum Factor, and Cortisone. (18739)

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Recent investigations have shown that relatively large amounts of dehydroisoandrosterone were interchangeable with pteroylglutamic acid (PGA) for growth of *Streptococcus faecalis* and *Lactobacillus casei*(1). In view of these observations cortisone was tested with *S. faecalis* for its ability to replace PGA and with *Leuconostoc citrovorum* for its ability to replace citrovorum factor (CF)(2), a substance related chemically to PGA(3). Cortisone was found to replace both PGA and CF for the two organisms mentioned; moreover, it reversed the inhibitory effects of Aminopterin, a potent antagonist for both PGA and CF.*

Experimental. The cultures used were *Le. citrovorum* ATCC 8081 and *S. faecalis* ATCC 8043. The medium used for *Le. citrovorum* was that of Sauberlich and Baumann (2) substituting acid-hydrolyzed casein for part of the amino acid mixture. The basal medium of Rabinowitz and Snell(5) was used for *S. faecalis*. The organisms were grown at 37°C, growth being observed turbidimetrically at 20 hours using a Coleman Model 11A spectrophotometer. Both the clinical aqueous suspensions and crystalline preparations of cortisone were used.† Leucovorin, a synthetic substance with CF activity(3), was used as a source of CF.

Results and discussion. Preliminary experiments indicated that the method of dissolving and diluting cortisone had a marked influence on the amounts required to give growth of the organisms. Table I shows the results obtained with a variety of sample treatments. The mild acid treatment was used to destroy any citrovorum factor possibly occurring in the samples of cortisone. No evidence for the presence of CF was found. The acid treatment consistently enhanced the potency of the clinical cortisone preparation while in 2 experiments the acid treatment decreased the potency of the crystalline cortisone sample.

The most reproducible results were obtained by evaporating alcoholic solutions of crystalline cortisone in the assay tubes. In several experiments by this technic 1.5 γ per ml gave

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* Preliminary experiments on replacement of PGA and CF by cortisone were discussed at Federation Meetings, 1951(4).

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† The clinical suspension was "Cortone Merck". A sample of crystalline cortisone acetate was obtained through the courtesy of Merck and Co.