TABLE I. Effect of X-Irradiation and Pooled Sera from X-Irradiated Embryos on Uptake of I^{131}.

Exp. groups	Age in days	Sample size	Counts/min. (mean value)	p*
Control I	12–13	66	7,414 ± 591†	
I (600 r)	"	106	10,195 ± 438	.005
Control I	15–16	37	17,278 ± 1205	
I (600 r)	"	45	21,380 ± 1512	.05
Control II	14–15	9	17,903 ± 3879	
II (pooled sera)	"	15	31,417 ± 3848	.05
Control III	15–16	16	16,125 ± 1528	
III (pooled sera)	"	10	23,701 ± 2053	.01

* Significant level for difference between means of treated and control calculated by means of Student's "t" test.
† Stand. error of mean.

in a similar increase in thyroidal uptake of I^{131}.

Conclusion. This finding gives direct evidence that the change in uptake of I^{131} by the thyroid following exposure of chick embryos to x-rays is indirectly mediated *via* a substance found in plasma after exposure.

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Specificity of Anamnestic Response in Chickens.* (24954)

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The phenomenon of anamnesis, as originally recognized by Solomonsen and Madsen (1) in 1896, referred to the secondary response to diphtheria toxin of animals previously immunized with the same antigen. However, the term "Anamnestische Serum Reaktion" was coined to describe observations of Conradi and Bieling(2), in 1916, that soldiers previously vaccinated with typhoid vaccine had a sharp rise in typhoid antibody titer during the course of unrelated febrile diseases. Since these early observations numerous workers have reported increases in antibodies to a primary antigen following secondary stimulation with unrelated antigen [*Cf.* reviews by Wilson(3) and Freund(4)]. Others, however, have been unable to demonstrate such secondary non-specific responses(3-5). Recently, Dixon and Maurer(5), using quantitative technics, have shown that rabbits previously immunized with one antigen accelerate and magnify the immune response to primary injection of a heterologous antigen, only if the 2 antigens are related. Furthermore, the secondary non-specific responses occurred only if the primary and secondary antigens were related. In attempts to define the specificity of the anamnestic response, the common fowl has been but little employed as antibody producer, despite the fact that the fowl has the advantage of reacting rapidly to single small injections of a variety of antigenic substances and producing antibodies in a relatively higher titer, reaching a peak in 7 to 9 days(6-8). It

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TABLE I. Circulating Antibodies following Primary and Secondary Injections.

Group	Primary antigen*	No. of chickens	1st heterologous inj. Antigen	1st heterologous inj. Response†	2nd heterologous inj. Antigen	2nd heterologous inj. Response
I	HGG	18	Crys-BSA	BSA		
II	Crys-BSA	21	HGG	HGG		
III	Powd-BSA	13	Powd-BSA	BSA & HGG		
IV	"	14	HGG	"		
V	"	7	"	"	Hemocy.	Hemocy.
VI	"	1	Hemocy.	Hemocy.		
VII	Crys-BSA & HGG	6	HGG	HGG		
VIII	"	1	Powd-BSA	BSA & HGG		
IX	HGG	10	"	BSA		
X	"	11	HGG	HGG		
XI	"	1	"	"	PSG	PSG
XII	HA	1	Thyrogl.	Thyrogl.	"	"
XIII	"	1	HA	HA	"	"
XIV	"	1	Thyrogl.	Thyrogl.	Hemocy.	Hemocy.
XV	Hemocy.	2	Crys-BSA	BSA	Crys-BSA	BSA
XVI	PSG	2	Hemocy.	Hemocy.		
XVII	Thyrogl.	10	Crys-BSA	BSA	Hemocy.	Hemocy.
XVIII	"	1	HGG	HGG		
XIX	"	1	Thyrogl.	Thyrogl.	PSG	PSG

* In all cases animals produced detectable antibodies to primary antigen(s) at 8 days following inj.

† No attempt was made to differentiate degree of response: thyroglobulin injections resulted in weakly flocculating reactions; all others reacted quite strongly.

would seem likely, therefore, that immune responses of the fowl might be profitably employed in further elucidating this problem of specificity of the anamnestic response.

Materials and methods. One hundred and twenty-two male New Hampshire Red and Arbor Acre White Rock cockerels, ranging in age from 5 to 8 months, were employed. Seven relatively pure soluble proteins were used as antigens: Human gamma globulin (HGG), Cutter Labs, Human serum albumin (HSA), Cutter Labs, Crystalline bovine serum albumin (Crys-BSA), Armour and Co., Powdered bovine serum albumin (Powd-BSA), Pentex, Inc., Hemocyanin (Hemocy.), from Keyhole Limpet,† Thyroglobulin (Thyrogl.), Warner-Hudnut, Pumpkinseed globulin (PSG), Difco Labs. A micro-diffusion agar technic(9) was used to test the specificity of hyperimmune sera against BSA and HGG antigens. The powdered BSA was shown to contain trace amounts of globulin and HGG contained trace amounts of albumin. The animals were divided into 19 groups and treated as described in Table I. In each case the primary injection consisted of a single intravenous injection of 40 mg of antigen/kilo body weight. The animals were bled 8 days following injection and the presence of antibody determined by flocculation tests. The sera of Groups I and II were also analyzed for antibody Nitrogen (AbN) by quantitative precipitin technic previously described(8). Four to 8 weeks later the chickens were injected with either an homologous or heterologous antigen and bled at various intervals following this second injection. In addition, several groups of birds received a third injection with a heterologous antigen at intervals of 4 to 8 weeks following secondary injection. Each injection was preceded by bleeding, which was tested for presence of antibodies to previously injected antigen(s).

Results. As shown in Table I, secondary injections of one or more antigens acted only as primary stimuli to the antigens used, and did not elicit antibodies to previously injected antigens except in those cases where a definite relationship between primary and secondary antigens was shown. A secondary injection of HGG following disappearance of antibodies to a primary injection of Powd-BSA caused reappearance of anti-BSA antibodies (Groups III, IV, V). It was also found that a secondary injection of Powd-BSA (Group VIII) following a primary combined injection of Crys-

† Generously supplied by Dr. D. H. Campbell, California Inst. of Technol.

BSA and HGG recalled antibodies to HGG.

Quantitative studies were carried out on Groups I and II to analyze the effect of previous immunization with one antigen on the primary antibody response of a heterologous antigen. To this end Group I was given HGG as primary antigen and Crys-BSA as secondary antigen. At 8 days following primary injection, all animals produced an average AbN titer of 493 μg/ml of undiluted serum (μg/ml serum). Bleedings made 5 weeks after this primary injection showed no detectable antibodies to HGG in the circulating system. Furthermore, the secondary injection of BSA did not elicit detectable renewed response to the primary antigen when tested at 4 and 8 days following heterologous injection. On the other hand, average AbN value of 582 μg/ml serum for the response to BSA approximated that of 479 μg following primary injection of BSA in Group II.

Group II, initially given a single intravenous injection of Crys-BSA, responded with an average AbN titer of 479 μg/ml serum at 8 days following primary injection. There were no detectable anti-BSA antibodies in the circulating system when these animals were tested at 4 to 5 weeks after primary injection. At this time they were given a secondary injection of HGG. When tested for presence of antibodies to BSA and HGG at 4, 5, and 8 days following secondary injection, this group showed no antibodies to the primary antigen. Furthermore, the average AbN value of 485 μg is approximately equivalent to average titer of 493 μg obtained following primary injection of HGG in Group I. Thus, the primary response to HGG was unaffected by previous immunization of the same chicken with BSA.

Groups I, II, VII, X, and XI which received HGG either as a primary or secondary injection might be expected to contain heterologous antibodies following secondary injection. However, they did not. This could conceivably be attributed to the exceedingly small amount of contamination of albumin in the HGG.

Discussion. The confusion regarding specificity of the anamnestic response has largely been due to use of such complex antigens as serum proteins(10), polysaccharides(11), erythrocytes(12), and bacteria(13). Furthermore, little attention was paid to the relationship between primary and secondary antigens as the use of such closely related substances as sheep and horse sera by Hektoen(14), and typhoid, paratyphoid, and dysentery bacilli by Tsukahara(15). In many cases the interval between primary and secondary injection was arbitrary and the animals were not tested for presence of antibody at time of secondary injection. Furthermore, most early workers used the rabbit as their experimental animal (3) which normally requires a series of injections over relatively long periods of time to induce significant antibody responses. Although multiple injections are usually effective in enhancing antibody titers, numerous studies(16,17) have shown that such procedures tend to reduce the specificity of antisera produced against both soluble and particulate antigens. The present study employed the chicken which reacts rapidly to single small injections of antigen to produce antibodies of relatively high titer. The antigens used were all relatively pure soluble proteins. All animals were tested for presence of antibody previous to secondary antigenic stimulation.

In no experiments was it possible to demonstrate a non-specific anamnestic response due to stimulation with one or more secondary antigens if this material showed no cross reactivity with the primary antigen. The reappearance of anti-BSA antibodies following a secondary injection of HGG subsequent to a primary stimulation with Powd-BSA was conceivably elicited by the albumin contaminant in the HGG. In the reciprocal experiment the recall of antibodies to HGG following injection of Powd-BSA subsequent to a primary injection with Crys-BSA and HGG could conceivably have been elicited by the gamma globulin contaminant in Powd-BSA. In either case, the contaminant in the second injection might reasonably be regarded as having acted as a secondary stimulus to the cross reacting component in the primary antigen. Furthermore, the quantitative antibody response to the secondary antigen was unaffected by previous stimulation of antibody mechanism with primary antigen as evidenced by comparison

with previously uninjected control groups.

Summary. 1) The effects of secondary injections of various soluble protein antigens, subsequent to disappearance from the circulating system of antibodies to a primary antigen, are described. 2) Secondary antigen injections do not elicit antibody responses to previously injected antigens, if the antigens are unrelated. 3) Quantitative levels of antibody response to secondary antigens are apparently unaffected by previous antigenic stimulations of the animal, if the antigens are unrelated.

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Nychthemeral Variations in Plasma Free Corticosteroid Levels of the Rat.* (24955)

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Measurements of "resting levels" of plasma corticosteroids in a series of experiments in the rat relating to CRF activity *in vivo*(1) suggested a diurnal rhythm in this index of adrenocortical function. If (diurnal) variations were as large as we suspected, it was important to obtain an accurate level/time relationship, and an exact estimate of extremes of these variations. No report on similar investigations has been found in the literature, owing probably to recent availability of methods to measure plasma corticosterone levels in the rat.

Materials and methods. A group of 100 animals (rats, males, Holtzman Farms) were housed in our animal quarters (illuminated with daylight only) 40 days prior to start of experiment. On day of experiment (Jan. 31 to Feb. 1), one animal at a time was taken from a different cage and was decapitated within 20 seconds. Sacrifice of animals (B.W. 200-230 g) was done outside animal quarters, at 12 noon, 4 p.m., 8 p.m., 12 midnight, 4 a.m., and 12 noon. Blood was collected in 50 ml beakers containing 0.01 ml of heparin solution 1/1000, and samples taken for hematological studies. Total white cell counts were done with routine laboratory methods. Air-dried blood specimens were stained with Wright-Giemsa solution, and 500 leukocytes identified for each differential count. The absolute number of eosinophils, neutrophils, lymphocytes, and monocytes/mm^3 of blood was calculated from total white cell and differential counts. Calculated eosinophil values determined in this manner are comparable to those obtained by direct counting methods (Higgins, G.M., personal communication).

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