The complement in normal serum of other species of animals is also inactivated by iodine. The animals studied include rat, hog, duck, man, dog, and chicken. The value of the ratio of complement to iodine for rat and hog have been found to be also constant (Table II).

Gordon, Whitehead and Wormall[1] reported that inorganic salts can inactivate complement. In order to show that the inactivation by iodine is not due to a specific effect of iodine or iodide, the inactivated serum was dialyzed to remove iodide and any remaining iodine. The serum so treated was found to possess no alexic activity. Therefore, the inactivation of complement by iodine must be a chemical reaction, most probably an oxidation. In fact, the results just reported indicate that there is a stoichiometric relationship between the amount of iodine consumed and the amount of complement inactivated. This indicates that the inactivation of complement proceeds so rapidly that the first portion of iodine added is almost completely reduced by complement and only the excess of the reagent is reduced by other substances in serum.

Our finding suggests a simple and rapid method of determining the amount of complement in guinea pig serum. The actual method and its significance will be reported in another communication.

Conclusions. 1. Complement in sera of different species of animal can be inactivated by iodine. 2. The amount of iodine necessary to inactivate a definite amount of complement in the serum of a species of animal, is a constant. 3. That constant varies in different species of animal.

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Effects of Chronic Injection of Rats with Extract of Pituitaries of the Same Species.

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Until about a year ago studies dealing with the production of loss of sensitivity to anterior pituitary extracts in animals subjected to chronic administration of these extracts had quite uniformly shown that this phenomenon apparently was one of species specificity. It appeared that the sera of such animals exerted a protec-

tive action in normal animals only against the active substances of the pituitaries of the species used for the chronic treatment.* These findings have recently been supported by the work of Brandt and Goldhammer,[1] Sulman,[2] and Zondek and Sulman.[3]

Within the past year, however, data have accumulated which do not support such specificity. Parkes and Rowlands[4, 5, 6] have reported that the serum of rabbits which have become refractory to the gonadotropic principles of the pituitary of the ox inhibited in other rabbits the gonad stimulating substances of the pituitaries of sheep, horse and the human, of the urine of pregnant women and of pregnant mares' serum. This antiserum as well as the antiserum to extracts of horse-pituitary inhibited in immature rats the action of extracts of human hypophyses. In this test animal, however, these antisera failed to inhibit the gonad stimulating principles of the pituitaries of the sheep and pig. Further, it was found that such antisera produced effects on the reproductive system of normal rabbits and rats[4, 6, 7] and the thyroids of normal guinea pigs[8] resembling those obtained by hypophysectomy. This is in line with earlier findings which indicate that the chronic administration of heterologous hypophyseal factors produces a neutralization of the animals' own pituitary hormones.

That the refractoriness to pituitary extracts may not be limited by species specificity is also indicated by work of Thompson and Cushing.[7, 8] These workers found that antisera to the gonadotropic and thyrotropic principles of sheep's pituitaries inhibited the corresponding active constituents of the pituitaries of other species and of certain types of human urine. The work of Gregerson, Clark and Kurzrok[9] also conforms with these findings.

Zondek and Sulman[3] maintain that the production of gonadotropic inhibitory substances manifests strict species specificity. By means of quantitative determinations they showed that the inhibitory effectiveness of the antigonadotropic factors is very much less against a heterologous gonadotropic factor than against the homo-

* For references see (14).

1 Brandt, R., and Goldhammer, H., *Z. f. Immunitaetsforsch.*, 1936, **88**, 79.

2 Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

3 Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 712.

4 Parkes, A. S., and Rowlands, I. W., *J. Physiol.*, 1936, **88**, 305.

5 Rowlands, I. W., *J. Physiol.*, 1937, **90**, 19P.

6 Rowlands, I. W., *Proc. Roy. Soc. London*, 1937, **B121**, 517.

7 Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. London*, 1937, **B121**, 501.

8 Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 637.

9 Gregerson, H. J., Clark, A. R., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 193.

logous one. They point out that in qualitative determinations where excessive quantities of antiserum are used this difference may not be apparent. Parkes and Rowlands[4, 5] have also observed that some antisera are more effective against their own antigenic extracts.

In connection with the question of species specificity the production of a refractory state in animals receiving pituitary hormones of homozoic animals is of importance. Most attempts to produce inhibitory substances in this manner have failed. Positive results have been obtained by Selye, Collip and Thomson[10] with rats and Collip[11] with sheep. Thompson[12] injected sheep with sheep-pituitary extract for a longer period than did Collip but failed to produce inhibitory substances. It is possible that the latter employed a more drastic procedure for the preparation of the extract which altered the antigenic properties of the active material.

In the present investigation we have attempted to produce a refractory condition in rats by the chronic administration of an extract of the pituitaries of the same species instead of implanting fresh anterior lobes as we had done previously.[14] This was done to determine whether an extract of the gonadotropic material of rats' hypophyses could be prepared which would react in a manner antigenically similar to active material of fresh anterior pituitary.

The anterior lobes, dissected from the posterior lobes of over 4000 rats, were desiccated by means of acetone. The dried glands were ground to a powder and weighed. Fresh extracts were prepared every 10-15 days by extracting a weighed portion of the powder with N/10 $NaHCO_3$, centrifuging and neutralizing the extract with N/10 HCl. Each rat was injected daily with an amount of extract equivalent to 2 anterior lobes.

The chronic administration of this extract was started on ten 55-60-day-old female rats. All animals remained in good health until they were sacrificed. At intervals between 107 and 255 days of treatment 2 of the animals were used in each test for refractoriness. Decapitation was performed under light chloroform anesthesia, the blood was combined and centrifuged and the serum used for the detection of gonadotropic inhibitory substances.

Table I contains the weights of some of the endocrine glands and the uteri (free of fluid) of these animals receiving the chronic

10 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

11 Collip, J. B., *Can. Med. Assn. J.*, 1937, **36**, 199.

12 Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 634.

14 Katzman, P. A., Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1937, **21**, 1.

TABLE I.
Effect of Pituitary (Rat) Extract on Adult Female Rats.

Injection period, days	Body wt. at autopsy, gm.	Ovaries, mg.	Uterus, mg.	Adrenals, mg.	Thyroid, mg.	Pituitary, mg.
107	172	568	580	37	16	11.1
107	168	228	726	34.5	10.5	9.2
133	205	613	705	50	18	10.3
133	198	286	440	31	20	16
184	215	155	—	47	22	13.6
184	208	200	—	44	16	10.2
241	210	215	546	51	16.5	9
241	245	320	516	60	21.5	17
255	269	190	1300	47	23	13
255	226	235	585	48	16.2	8
Aver.	212	301	675	45	18	11.7

treatment. The ovaries of 2 animals sacrificed after injection periods of 107 and 133 days respectively, were very much larger than those of the other animals. This probably was due to an unusual sensitivity since there was no progressive decrease in the weights of the ovaries of the other animals injected for longer periods. Even after 255 days of treatment the ovaries were still 400-500% larger than those of untreated controls. They were loaded with large corpora and follicles of various sizes which, histologically, appeared to be functional. The weights of the other endocrine glands did not differ significantly from those of untreated animals. The uteri, of course, were markedly enlarged.

The serum was tested for antigonadotropic activity in 21-day-old female rats by determining whether it would diminish the effect of the extract of rats' pituitaries. Each animal received a total of 2.1 cc. to 3.0 cc. of the serum administered subcutaneously twice daily on 3 successive days. The injection of the extract was begun one day after the first injection of serum. Each immature female received daily an amount of extract equivalent to 2 rats' pituitaries, administered in 2 subcutaneous injections for 3 successive days. The serum and extract were injected in widely separated sites to prevent any influence of the former on the absorption of the latter. Controls, receiving only the extract, were used for each test. All animals were killed 100 hours after the first injection of the extract, and the ovarian weights determined.

It is evident from Table II that in no case was there a significant inhibition of the response to the extract. In some instances we have observed an augmentation similar to that noted in our earlier work with fresh anterior lobe implants. It is interesting to note that this augmentation was manifested by the sera obtained from the rats which were treated for 107 to 133 days but not by the sera

TABLE II.
Influence of Serum of Chronically Treated Rats on Effect Produced by Injection of Pituitary (Rat) Extract in Immature Female Rats.

Treatment	No. of rats used	Body wt. (gm.)			Wt. of ovaries (mg.)		
		Min.	Max.	Aver.	Min.	Max.	Aver.
107-day serum + extract	4	39	43	41	36.5	46.3	41.8
Extract alone	4	35	44	41.8	20.3	31.3	23.7
133-day serum + extract	3	40	46	42.1	29.0	60.5	50.0
Extract alone	2	38.5	40	39.2	19.5	31.0	25.2
184-day serum + extract	5	40	46	42.6	14.9	55.7	35.5
Extract alone	5	36	43	39.2	16.8	38.7	29.6
241-day serum + extract	5	38	45	42	23.0	38.0	32.6*
Extract alone	5	32	37	34.8	31.0	46.0	36.9
255-day serum + extract	5	40	55	47.8	29.0	89.0	49.0
Extract alone	5	40	50	43.2	24.0	78.0	50.0

*An extract prepared from a different sample of powdered anterior lobes was used for this group and the succeeding groups of animals.

obtained from the animals treated for longer periods. Similar observations have been made by Collip[11] and Thompson.[13] We are at present studying the time of appearance and the nature of this augmentory substance.

It is unfortunate that a larger number of immature rats could not be used in this investigation, since the response to the extract was quite varied. However, owing to the large number of animals required to supply the hypophyses for the chronic treatment, the amount of such serum obtainable is small.

Summary. We have confirmed our previous findings that rats receiving chronic treatment with active principles of the anterior lobes of the same species fail to become refractory. Under the conditions of the experiment an aqueous extract of acetone-desiccated hypophyses obtained by extraction with dilute sodium bicarbonate produced effects similar to those of implants of fresh anterior lobes.

13 Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 640.