TABLE I. Serological and Bacteriological Studies on Mice Infected with Strain 19, *Brucella abortus* and Treated with Saline or Cortisone.

No. mice & days after infection sacrificed	Saline-treated				Cortisone-treated			
	Reciprocal of brucella agglutinins (avg)	Avg No. of brucella colonies per 0.1 mg of organ*			Reciprocal of brucella agglutinins (avg)	Avg No. of brucella colonies per 0.1 mg of organ*		
		Liver	Spleen	Kidney		Liver	Spleen	Kidney
5 in each group in 7 days	320	2+ to 3+	4+	3+ to 4+	640	3+ to 4+	4+	
5 in each group in 15 days	1280	40 (1 neg.)	2,500	175 (2 neg.)	1280	2,550	5,500	4,800 (1 neg.)
10 saline & 8 cortisone, 21 days	1280	39 (1 neg.)	2,950	62 (3 neg.)	640	189 (1 neg.)	10,900	294 (1 neg.)

* 2+ = more than 5,000 colonies; 3+ = more than 50,000 colonies; 4+ = solid growth of brucellae.

hepatic granulomas. Fewer granulomas were apparent in livers of the cortisone-treated mice, and they were less well defined. Fifteen days after infection, cellular infiltration in the 2 groups was approximately the same, except that the saline-treated group had more organized granulomas in the liver. After 3 weeks well organized granulomas were noted in livers of both groups of mice, but the number of granulomas in the cortisone-treated group was slightly greater than in the saline-treated mice. In general, the granulomatous tissue reaction of the saline-treated group of infected mice was more uniform than in the cortisone-treated mice. No granulomas were seen in the cortisone-treated, non-infected control mice, but a few areas of cellular infiltration were observed.

Summary and conclusions. An acute infection with strain 19, *Br. abortus* was established in mice. Simultaneous treatment with cortisone enhanced the infection, but did not interfere with the formation of brucella agglutinins. However, cortisone did not cause the severe necrosis of the hepatic parenchyma and proliferation of brucellae that had been previously observed in studies with a more virulent species of Brucella.

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Evidence for Existence of Mammogenic Hormone.*† (24387)

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For over 20 years it has been known(1) that the anterior pituitary gland is necessary and directly or indirectly responsible for mammary gland lobule-alveolar growth. Whether a specific mammogenic (mammary gland growth promoting) hormone is secreted by the anterior pituitary has not been answered conclusively(2,3). Much disagreement has stemmed from the fact that mammogenic activity has been found to be associated with those extracts of pituitary tissue containing high lactogenic activity. Comparisons of lactogenic and mammogenic potencies of various preparations(5) indicated extreme differences in relative amounts of these hormones. However, these assays were based on older, more subjective technics(4,5) which had no method of estimating variance of assayed potency. If definite, significant differences were shown between lactogen/mammogen ratios of several anterior pituitary preparations it would appear that mammary gland growth is not due

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TABLE I. Lactogen/Mammogen Ratios of Various Pituitary Fractions.

Substance	Lactogen mean log, relative potency	Mammogen mean log, relative potency	Lactogen/mammogen ratio
Anterior pituitary	−2.79588 ± .16997	−.58021 ± .22325	−2.21567 ± .280
Initial extract	− .78252 ± .12617	−.67346 ± .13906	− .10906 ± .187
Armour lactogenic hormone, lot 759-014A (mammogen assay 1)	− .91721 ± .23915	.94768 ± .21472	−1.86489 ± .321
Idem (mammogen assay 2)	− .91721 ± .23915	1.10023 ± .09285	−2.01744 ± .256
Armour lactogenic hormone lot R-491154	.31702 ± .24961	1.09899 ± .08473	− .78197 ± .264
Squibb lactogenic hormone lot 71713	.41196 ± .0921	1.11136 ± .0827	− .6994 ± .124

to the lactogenic hormone *per se*, but rather to a separate pituitary hormone. It will be shown that the amount of lactogen and mammogen in several preparations differs widely, indicating that the pituitary hormone stimulating mammary gland growth is different from the hormone stimulating milk secretion.

Materials and methods. The lactogenic hormone was assayed by a method developed by Grosvenor and Turner(6) based upon the response of pigeon crop glands to graded doses of sheep lactogenic hormone. One dose each of desiccated anterior pituitary powder, initial extract of anterior pituitary(7), Armour lactogenic hormone (lot 759-014A), Armour lactogenic hormone (lot R-491154) and Squibb lactogenic hormone (lot 71713) was injected intradermally over the crop-sac into different groups of pigeons each day for 4 days. On the fifth day the birds were killed, crop-sacs removed and cut in half. Each half was stretched fully by 2 persons over a light source while a third person matched the area of response as closely as possible with one of 8 plastic gauges which ranged in diameter from 0.50 to 4.0 cm. Relative potency was calculated from mean response and standard regression line(6). Standard error of assay was calculated as standard error of difference between means of samples (S^2 assay = Syx^2 + S^2 sample), where Syx is the standard deviation about the line of means(9) and S sample is the standard error of the sample. Desoxyribosenucleic acid (DNA) method of determination of extent of mammary gland growth was used. The previously determined synergizing dose of estradiol benzoate (.75 μg/day) with graded levels of progesterone and length of injection time (10 days) were used to produce a standard DNA line in each set of assays(8). Comparisons of growth were made by injecting .75 μg estradiol benzoate plus single doses of each of the above pituitary preparations; one replication with Armour lactogenic hormone (lot 759-014A) was carried out. Mammogenic potency and error limits were calculated in essentially the same manner as the lactogenic hormone potency calculations. Since distributions about the relative potencies are normal only when in log form, results of assays are reported as mean log relative potencies, with log standard errors. Log lactogen-mammogen ratio was computed as the difference between mean log relative potencies of any given preparation. Standard error of the ratio was computed in same manner as standard error of mean difference.

Results. Highly significant differences were found between lactogen/mammogen ratios of several anterior pituitary preparations assayed (Table I). Dessicated anterior pituitary differed in ratio-potency from initial extract, Armour lactogenic R-491154 and Squibb lactogenic 71713. Initial extract differed from the 3 purified lactogenic preparations. Armour lactogenic 759-014A, on both mammogen assay and reassay differed from the other 2 lactogenic preparations. No significant differences were found between desiccated anterior pituitary preparation and Armour lactogenic lot 759-014A, nor between Armour lactogenic R-491154 and Squibb lactogenic lot 71713.

Discussion. Comparisons of the ratio of lactogen/mammogen biological activity in a series of anterior pituitary preparations varying in purity from an acetone dried powder to

preparations showing high lactogenic activity confirm earlier observations(5) indicating marked differences. In the present study improved objective technics for the assay of these hormones and statistical procedures were used.

In consideration of results of screen assays of various substances, several factors must be taken into account. The true slope of lines which might be produced by graded levels of the "unknown" substance could easily differ from that of the standard, indicating a different mechanism of action of the substances in question. According to present theory, the substances compared for mammogenic activity probably do have mechanism of action different from the standard against which they were compared (progesterone). The converse logic cannot be applied in this case, however. One cannot assume that the substances have the same mechanism of action if a difference in slope is not found between the "standard" and "unknown."

Confidence intervals themselves should be taken with some reservations, keeping in mind that they are only estimates of the true variance.

In view of the fact that screen assays are "pilot" in nature, to differentiate between activity or no activity (and to a certain degree the relative amount of activity), some criteria must be used in the assay for this purpose. In mammogenic assay, whole mount observation of ¼ of the total mammary gland tissue gives some indication of type and extent of growth. If consistent normal alveolar growth is found upon examination, and the mean DNA value falls within the range of DNA produced by respective progesterone doses, it would appear reasonable to interpolate an equivalent progesterone dose and subsequent relative potency. If the DNA values of the "unknowns" fall outside the standard range, and whole-mount observation indicates other than normal development, it would seem prudent to adjust the dose of "unknown" accordingly on a reassay, rather than make any attempt at extrapolation.

Relatively conclusive proof has been presented that the lactogen/mammogen ratios are different in several of the preparations studied. With this evidence at hand, it would be difficult to reconcile a theory proposing that lactogenic and mammogenic hormones are the same entity.

While definite differences in lactogen/mammogen contents between several anterior pituitary preparations have been demonstrated in the present and previous work(5), ultimate proof of existence of a specific mammogenic hormone of the anterior pituitary will rest upon isolation of a factor essentially free of lactogenic hormone but exhibiting high mammary gland growth promoting activity.

Summary. Improved objective technics for assay of lactogenic and mammogenic hormones have been developed. The amount of lactogen and mammogen has been estimated in several different pituitary preparations by these assay methods. Wide differences in lactogen/mammogen ratios between preparations were demonstrated. These observations are taken to indicate that the pituitary hormone stimulating mammary gland growth (mammogen) is different from the hormone stimulating milk secretion (lactogen).

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