Embryomegaly and Increased Fetal Mortality in Pregnant Rats with Mild Alloxan Diabetes

Fredric Solomon, M.D., Chicago

INTRODUCTION

Pregnancy in diabetic women is associated with a high incidence of late intrauterine death of the fetus and perinatal mortality of the newborn. The birth weight of infants born of diabetic mothers is usually greater than that of infants born of normal mothers. The causes of the embryomegaly and high stillborn rate are not known. Attempts to reproduce experimentally clinical observations associated with diabetes in human pregnancy have had controversial and unsatisfactory results.[1-8]

Alloxan has been used for the past fifteen years to produce in experimental animals a condition analogous to human diabetes. Damage to tissues other than the β cells of the pancreas generally disappears in a few days if the animal survives.[9-12] Although transient lesions in the renal tubules have been frequently noted in alloxanized rats, Cohen[13] has shown that glycosuria and polyuria parallel the blood sugar levels in such animals.

In the experiments to be presented in this paper, diabetes of various degrees of severity was induced by the administration of alloxan to rats at various stages of gestation. When a mild form of diabetes resulted, there was a significant increase in the average fetal birth weight and an increased incidence of stillborn young. In most cases, severe diabetes resulted in early interruption of pregnancy, often preceding maternal death. Preliminary experiments in which fetuses were analyzed for moisture, protein and fat content suggest that offspring of mildly diabetic rats are large by virtue of true growth.

METHODS AND MATERIALS

A. *For effect of alloxan diabetes on pregnancy*

Fifty-three virgin albino rats of the Sprague-Dawley strain were mated with males when the females were three to four months old and weighed 204 to 233 gm. The presence of sperm on vaginal smears indicated the beginning of gestation. For the duration of the pregnancy, they were kept in individual wire-bottomed cages from which twenty-four-hour urine samples were collected for qualitative glucose determinations with Clinitest reagent tablets (copper sulfate and heating agent—Ames). The rats were allowed to eat a regular diet (Rockland Rat Diet) and drink tap water ad libitum. The animals were weighed and inspected daily.

Alloxan in 5 per cent aqueous solution was injected subcutaneously in doses of 67 to 125 mg. per kg. of body weight. In some animals, additional subcutaneous injections of 34 to 78 mg. per kg. were given after the initial single dose had failed to produce glycosuria. Eleven rats served as untreated pregnant controls.

Individual fetal weights were taken immediately after delivery, before nursing or cannibalism could take place.

B. *For comparison of composition of fetuses*

1. Some litters were used in the fetal analysis study. For purposes of convenience, analysis was carried out on four groups of fetuses in *each* litter: stillborn males, stillborn females, liveborn males, liveborn females. Groups containing more than six fetuses were subdivided. Immediately following delivery the fetus groups were weighed to the nearest 0.01 gm., wrapped in aluminum foil and placed in a freezer at —25° C. This, naturally, killed the living young; the fetuses were stored in this way for one to three months.

2. At the beginning of the analytic procedures on it, a fetus group was allowed to thaw at room temperature until moisture had ceased to condense on it and the weight of the group lay within ±0.10 gm. of the original neonatal weight. This "thawed weight" was used in later calculations.

3. Each group was then dried in a 100°-C. oven for ten to fourteen days, until the weight loss over a twelve-hour period became less than 1 mg. The percentage which moisture constituted of each group was calculated from these "dry weight" values. It was found

---

Winner of the 1957-58 Medical Student-Intern Essay Contest of the American Diabetes Association for the best paper in the field of diabetes reporting original work, whether laboratory investigation or clinical observation. This is his prize-winning paper.

From the University of Chicago School of Medicine, Chicago, Illinois. Present address (until July 1959): University of Illinois Research and Educational Hospitals, 840 South Wood Street, Chicago 12, Ill.

that groups which were analyzed directly from the birth room, i.e., fetuses which did not go through the freezing and thawing, had percentage moisture values nearly identical with those of their frozen litter mates.

4. For the other determinations, duplicate aliquots of a homogenate of each group were used. The homogenate of a group was prepared by hydrating the dried carcasses and placing them in a high speed homogenizer (*Waring Blendor*) for two hours. The homogenization was interrupted several times to "wash down" sprayed pieces of carcass back into reach of the blades. The homogenate was finally transferred to a graduated cylinder and diluted to a convenient, recorded level—generally equal to about 100 ml. homogenate per each fetus in the group.

5. One milliliter aliquots were removed from each homogenate for determination of percentage composition of protein. Total nitrogen content in each aliquot was determined by standard micro-Kjeldahl technic. The milligrams of protein in each sample were then calculated by multiplying the milligrams of nitrogen by the factor 6.25. The percentage of protein per group was then calculated.

6. For measurement of crude fat, 10 ml. aliquots of homogenate were measured into glass weighing bottles and dried in the 100°-C. oven. The residue was then simply extracted with anhydrous ether at room temperature for thirty-six hours, resulting in recovery of "ether-extractable substance" or crude fat. Only weighing bottles with glass covers and ground glass connections were used. When compared on aliquots of the same homogenate, this method yielded virtually identical results with other methods of fat extraction. No advantage was seen, therefore, in using a 3:1 alcohol-ether mixture or a heated reflux system.

7. Mineral ash determinations were done on a few groups by heating the dry residues of 10 ml. aliquots in a 550°-C. oven for four hours. The noncombustible remainder was weighed and used in the calculation of the percentage ash per group.

8. These methods of analysis are official methods for meat analysis of the Association of Official Agricultural Chemists.[14] The technic is, in general, similar to that of Salter and Best.[15]

RESULTS

A. *Normal pregnant rats*

The pregnancies of eleven untreated control animals are characterized by the data in table 1. A total of 109 young, weighing an average of 5.75 gm., were delivered after twenty-one or twenty-two days of gestation. Four fetuses (3.7 per cent) were born dead. The weight increment of the maternal tissue (equals difference between the body weight of the mother shortly after parturition and the initial body weight on the day of conception, expressed in percentage of the initial body weight) showed much variation. The weight increment was 14.6 per cent, on the average, with a range of 6.1 per cent to 23.6 per cent. There were no abortions or maternal deaths.

The necessity of obtaining control values simultaneously with experimental data is quite marked in this field, where gestation time, stillbirth rate, and average fetal weight vary significantly between different inbred strains.[3,7,35,39]

B. *Mild alloxan diabetes in pregnant rats*

Mild alloxan diabetes was induced in seven rats on the eleventh day of gestation (Group A) and in sixteen rats on the fourteenth day of gestation (Group B). The doses of alloxan in the initial subcutaneous injection varied between 67 and 80 mg. per kg. of body weight. In eight animals one or two additional injections of 34 to 78 mg. per kg. were given on the following days.

The mild diabetes was characterized by slight polyuria and constant excretion of 0.5 to 2 per cent glucose into the urine. Four animals showed glucose-free urine a few days after delivery but the other nineteen rats became permanently diabetic. All animals remained in good condition, gained weight and lactated. The average weight increment of maternal tissue was 15.8 per cent, which does not differ significantly from the control value.

The effect of mild alloxan diabetes on fetal body weights at birth and on the incidence of late fetal death is shown in table 1. A significant increase of the average fetal birth weight was observed in both groups of rats with mild alloxan diabetes as compared with control values. The difference is shown graphically in figure 1. The stillbirth rate is increased nearly sevenfold over control values in Group B, and it is 2.8 times the control rate in Group A. The latter difference, however, is barely outside the limits of statistical significance ($p=0.06$). In these animals, as well as in the controls, stillborn fetuses showed moderate variability in body weight, but, when averaged, did not display a weight different from that of their respective Groups; they are not reported separately.

In the past, experimenters have produced oversized newborn by inducing a postmature state in prolonging gestation[16,17] or by inducing a reduction of the number of young per litter.[18] It is felt that neither situation existed in the mildly alloxan diabetic series reported

TABLE 1

The effect of mild alloxan diabetes on the pregnancy and newborn of rats

	Normal controls	Group A alloxan on 11th day	P versus controls	Group B alloxan on 14th day	P versus controls
1. Number of litters	11	7		16	
2. Number of young (av.) per litter	9.9	11.0		10.1	
3. Gestation time (days)	21.6±0.185*	21.7±0.184*	>0.05 t test	22.1±0.125*	<0.05 (=0.02) t test
4. Total number of young	109	77		162	
5. Liveborn young	105	69	>0.05 (=0.06) x^2 test	121	<0.01 x^2 test
6. Stillborn young	4	8		41	
7. Stillbirth rate (per cent)	3.7	10.4		25.3	
8. Average weight of all fetuses (gm.)	5.75±0.07*	6.10±0.19*	<0.01 t test	6.39±0.09*	<0.01 t test
9. Average weight of male young	5.85	6.24		6.54	
10. Average weight of female young	5.63	5.95		6.20	

*Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

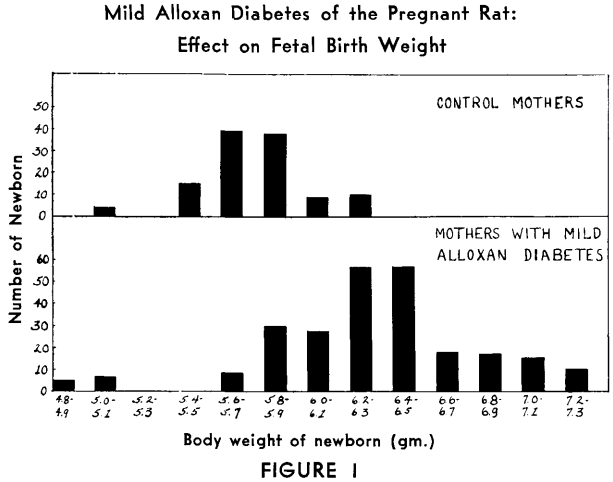

FIGURE 1

here. The number of fetuses per litter is, if anything, greater than in the control rats. On the other hand, it is true that on the average the offspring of Group B spent half a day longer in utero than offspring of controls (see line 3, table 1). However, on the basis of control values in our laboratory, it is considered "normal" for this strain of rats to deliver on either the twenty-first or twenty-second day of gestation; thirteen of sixteen Group B rats did so. The three litters which were delivered on the twenty-third day of gestation contained twenty young weighing an average of 6.27 gm., which is below the Group's average of 6.39 gm. There were three stillbirths (15 per cent). Furthermore, there is a 10 per cent probability ($x^2 = 2.71$) that three gestations of twenty-three days could have occurred by chance.

C. *Severe alloxan diabetes in pregnant rats*

Severe alloxan diabetes, characterized by excessive polyuria and pronounced glycosuria, was induced in twelve pregnant rats on the third to eighth day of gestation (Group C) and in seven animals on the eleventh to sixteenth day of gestation (Group D). The dosage of alloxan in the initial injection ranged between 70 and 125 mg. per kg. of body weight. In six animals an additional dose of 40 to 70 mg. per kg. was injected on the following day. Very little gain in maternal weight was observed, and in several animals there was a profound weight loss.

No living young were produced by the rats with severe diabetes induced by alloxan administration early in pregnancy. Eight of the twelve animals in Group C died before the expected date of delivery, five to seven days after the diabetes was induced. Prior to each maternal death, there were clinical signs of abortion, and at autopsy total or partial reabsorption of all fetuses at their placental sites was found. One rat died undelivered on the twenty-first day of gestation. Its well-developed fetuses weighed 4.9 to 5.2 gm. and showed signs of

maceration. Three Group C rats carried their pregnancies beyond the twenty-second day of gestation. Two of these rats delivered fifteen dead young weighing 4.9 to 5.6 gm. on the twenty-fourth and twenty-fifth day respectively. The third rat delivered eight dead malformed young on the twenty-seventh day after conception.

Severe alloxan diabetes induced late in pregnancy did not cause maternal death, but there was early intrauterine death and reabsorption of fetuses in four of the seven animals in Group D. The abortions occurred five days following the induction of the severe diabetes. Three rats delivered only living young on the twenty-third, twenty-fourth and twenty-fifth day after conception respectively; the average body weight at birth of the twenty fetuses was 5.36 gm. (range: 5.15 to 5.80 gm.). All of these newborn died during the first days of extrauterine life, since the severely diabetic mothers did not lactate.

D. *Analysis of fetuses from control and mildly alloxan diabetic rats*

For this preliminary experiment, two litters from normal rat mothers were compared with two litters from rats with mild alloxan diabetes. The results of analysis for percentage composition of moisture, protein and fat are summarized in table 2. The number of determinations is clearly inadequate for statistical comparison, but inspection of these early results lends the impression of there being no demonstrable significant difference between the respective compositions of normal and experimental offspring. Such data suggest that the young of mildly alloxan diabetic rat mothers are large by virtue of proportional true growth, rather than by edema or excessive fat deposition.

It will be noted that when water, fat and protein determinations are combined, less than 97 per cent of total body weight for each group is accounted for. The remainder is composed in part of mineral ash (2.0 to 2.5 per cent), which was determined in a few groups but not throughout because of inconvenience. Of course, there is also a combined error of at least 1 per cent in the whole procedure.

DISCUSSION

The main purpose of the experiments reported in this paper was to find suitable experimental conditions with which to produce oversized fetuses in alloxan diabetic rats. Hultquist[7] reported that some litters from rats depancreatized at various stages of pregnancy contained *gigantic fetuses. Some large young from alloxan diabetic* rats were similarly observed by Bartelheimer and Kloos.[8] Most of the gigantic fetuses in both experiments were stillborn.[7,8] H. C. Miller, on the other hand, found no abnormalities in ten pregnancies of rats with pre-existing mild alloxan diabetes.[8] Friedgood and A. A. Miller injected pregnant rats with alloxan four days prior to parturition and noted no effect.[1]

In our own experiments, mildly alloxan diabetic rats excreting 0.5 to 2 per cent glucose into the urine and remaining in good condition delivered young weighing significantly more than normal controls. The stillbirth rate in these rats is similar to the 18 per cent rate found in the alloxan diabetic rats of Barns[6] and is comparable to the 15 to 30 per cent stillbirth rate in human diabetics.[19-23] Our stillborn fetuses were not necessarily larger than their litter mates.

Several workers have implicated maternal hyperglycemia as at least partially responsible for the abnormal size and fragility of newborn of human diabetics.[20,24-26]

TABLE 2

Chemical comparison of fetuses from rat mothers with mild alloxan diabetes with fetuses from normal rat mothers

	Number of fetuses	Average fetal weight (gm.)	Moisture content (per cent)	Protein content (per cent)	Fat content (per cent)
a. Diabetic mothers, Liveborn	11 (4 groups)	5.81 (5.22—6.96)	85.8 (85.7—86.0)	10.0 (9.91—10.2)	.705 (.562—1.09)
b. Diabetic mothers, Stillborn	10 (4 groups)	6.63 (5.37—7.07)	85.9 (85.3—86.1)	9.83* (9.52—10.9)	.693 (.614—.870)
c. Diabetic mothers, live and dead combined	21 (8 groups)	6.20	85.8	9.95*	.699
d. Normal mothers, All live	23 (5 groups)	4.98 (4.66—5.42)	87.4 (87.3—87.4)	8.64 (7.96—10.1)	.668 (.493—.859)
e. Difference between c and d		+1.22	−1.6	+1.31 or +0.19 gm. per fetus	+0.021

*These values do not include a group of five dead females containing 4.02 per cent protein. The average fetal weight, moisture and fat content of this group fall well within the stated ranges for those determinations.

The many reports of hypertrophy and hyperplasia of the islets of Langerhans in stillborn young of diabetic mothers[27-29] along with the frequent clinical finding—indeed, danger—of hypoglycemia in the liveborn offspring of diabetic mothers[19,30] have suggested that these young develop with chronic intrauterine fetal hyperinsulinism. Salter and Best[15] have described the growth-promoting properties of insulin. Hultquist reported increased islet tissue in his gigantic fetuses from depancreatized rats.[7]

On the other hand, Potter et al.[31] reviewed autopsies from several centers and cast serious doubt on the significance of the finding of proliferated fetal islet tissue. Furthermore, it has been shown that both fetal and neonatal mortality[32,33] and birth weight[34,35,28] are significantly elevated in prediabetic women, when maternal hyperglycemia is not demonstrable. Given et al.[21] present evidence that rigid control of the blood sugar in pregnant diabetics does not lower the stillbirth rate and does not reduce the incidence of large babies, as compared to nonacidotic, hyperglycemic women.

Barns[6,18] and Gilbert[36] have suggested that overproduction of maternal growth hormone causes fetal gigantism in prediabetic and diabetic women. In 1935 Watts[37] injected growth hormone into pregnant rats and produced large fetuses without an accompanying reduction of the number of fetuses per litter. Barns[18] and others[38] have been unable to duplicate these results.

In addition to factors in the maternal environment, a genetic element in embryomegaly has been introduced by Jackson,[23] who reported greater than normal birth weight in offspring of diabetic fathers. Prediabetic and diabetic women have even larger babies, however, and an elevated stillbirth rate not found in the diabetic father group. In our experiments, of course, it was possible to produce large fetuses in the absence of predisposing hereditary factors from either parent.

Hoet[24] has claimed that adrenal hypercorticalism during pregnancy plays an important role in fetal nutrition. Cortisone administered in small doses to pregnant rabbits favored passage of glucose from mother to fetus —thereby "activating" fetal growth, according to Hoet's view. On the other hand, Davis and Plotz[39] found normal fetal growth, inhibited maternal growth and an elevated stillbirth rate in nondiabetic pregnant rats treated with intramuscular cortisone, 3 mg. per day. No effects were observed with lower doses. It has been found in our laboratory that there is no additional increase in the embryomegaly or high stillbirth rate of mildly alloxan diabetic rats when they are given 3 mg. of cortisone per day—i.e., cortisone has no demonstrable effect on the findings reported here in table 1.[40]

The adrenal cortex enlarges and apparently increases its function in rats made diabetic with alloxan.[41-43] Since in the experiments of Davis and Plotz[39] the administration of cortisone has consistently resulted in increased fetal mortality, it seems possible that increased adrenal cortical activity in rats made alloxan diabetic during pregnancy might account for the lethal effect on some offspring of such animals. Field[44] in acute experiments has prevented adrenal cortical hypertrophy by regulating the alloxan diabetes with insulin. It would seem that experiments are called for in which the pregnancies of adrenalectomized and alloxanized animals are observed, with and without insulin treatment.

In addition to the increased growth of the islets of Langerhans noted above, other variable characteristics of large young from human diabetics which have been reported include: edema, excessive fat deposition, hyperplasia of various endocrine organs, increased bone length, and splanchnomegaly.[20,27,29] Preliminary results of our fetal analyses tend to associate only proportional "true" growth with the embryomegaly in young of mildly alloxan diabetic rats.

It must be noted that the very great abortion rate of the *severely* diabetic animals in our experiments is consistent with the work of Davis, Fugo and Lawrence;[2] the impairment of lactation confirms the reports of Sinden and Longwell[4] and Barns, et al.[6] There is a discrepancy, however, between our results and those of others[4,5] who report successful termination of normal pregnancies in rats with permanent severe diabetes which was induced with alloxan before mating. It seems possible that the initial phase of the severe diabetic state may be more deleterious than the subsequent phase for the maintenance of pregnancy in the rat.

Alloxan is a small molecule capable of crossing the placental barrier. One might ask, then, "Are these changes in fetal growth and viability the result of some direct action of alloxan on the developing embryo?" A definitely negative answer to this question would be possible only after extensive confirmation of Hultquist's report on the offspring of depancreatized rats.[7] However, it may be noteworthy that in the course of our experiments several animals died twenty-four to forty-eight hours after receiving the initial injection of alloxan; although these rats showed gross signs of toxic damage to liver and kidney, their intrauterine contents had remained intact.

SUMMARY

Alloxan diabetes of varying degrees of severity was induced in rats at various stages of gestation. Mildly

diabetic rats delivered young with birth weights significantly larger than control values; an increased incidence of stillborn young was also observed. Severe diabetes caused early interruption of pregnancy in most cases, and no large fetuses were delivered by these rats.

Gross chemical analysis was performed on a small number of fetuses from mildly diabetic rat mothers. Virtually the same proportions of moisture, protein and fat were found in these fetuses as were found in offspring of normal rats.

Several proposed explanations of the embryomegaly and high fetal mortality associated with pregnancies of human diabetics are discussed, in light of this experimental work and that of others.

SUMMARIO IN INTERLINGUA

Embryomegalia E Augmento Del Mortalitate Fetal In Rattas Pregnante, Levemente Diabetic Per Alloxano

Diabete de varie grados de gravitate esseva inducite per medio de alloxano in rattas a varie stadios del gestation. Rattas levemente diabetic parturiva juvenes con pesos natal significativemente supra le valores de controlo. Un augmento del numero de morte-natos esseva etiam observate. Diabete sever causava le precoce interruption del pregnantia in le majoritate del casos. Le fetos parturite per iste rattas non esseva anormalmente grande.

Grossier analyses chimic esseva effectuate in un micre numero de fetos ab rattas-matre levemente diabetic. In iste fetos, quasi le mesme proportiones de humiditate, proteina, e grassia esseva trovate como in le prole de rattas normal.

Es discutite—in le lumine de iste experimentos e del experimentos de altere autores—plures del explicationes proponite pro le embryomegalia e le alte mortalitate fetal que es associate con pregnantia in diabeticas human.

ACKNOWLEDGMENT

This work has been carried out under a grant of the Douglas Smith Foundation for Medical Research.

The author gratefully acknowledges the guidance and sponsorship of Drs. E. Jürgen Plotz and M. Edward Davis of the Department of Obstetrics and Gynecology, the University of Chicago and Chicago Lying-in Hospital, Chicago, Illinois.

REFERENCES

[1] Friedgood, E. C., and Miller, A. A.: Proc. Soc. Exp. Biol. and Med. 59:61, 1945.

[2] Davis, M. E., Fugo, N. W., and Lawrence, K. G.: Proc. Soc. Exp. Biol. and Med. 66:638, 1947.

[3] Miller, H. C.: Endocrinology 40:251, 1947.

[4] Sinden, J. A., and Longwell, B. B.: Proc. Soc. Exp. Biol. and Med. 70:607, 1949.

[5] Levi, J. E., and Weinberg, T.: Proc. Soc. Exp. Biol. and Med. 72:658, 1949.

[6] Barns, H. H. F., Linden, O., Morgans, M. E., Reid, E., and Swyer, G. I. M.: Lancet 1:841, 1950.

[7] Hultquist, G. T.: Acta Pathol. Microbiol. Scand. 27:695.

[8] Bartelheimer, H., and Kloos, K.: Zschr. Ges. Exp. Med. 119:246, 1952.

[9] Dunn, J. S., and McLetchie, N. G. B.: Lancet 2:384, 1943.

[10] Bailey, C. C., Bailey, O. T., and Hogan, W. H.: Am. J. of Med. Sci. 208:450, 1944.

[11] Houssay, B.: Canadian M. A. J. 56:519, 1947.

[12] Lukens, F. D. W.: Physiol. Rev. 28:304, 1948.

[13] Cohen, E. M.: Acta Physiol. Pharmacol. Neerl. 2:349.

[14] Official Methods of Analysis of the Ass'n. of Official Agricultural Chemists, published by the A.O.A.C., Washington, D.C., 1950.

[15] Salter, J., and Best, C. H.: Brit. Med. J. 2:353, 1953.

[16] Hoopes, E. C.: Proc. Soc. Exp. Biol. and Med. 31:1115.

[17] Snyder, F. F.: Bull. Johns Hopkins Hosp. 54:1, 1934.

[18] Barns, H. H. F., and Swyer, G. I. M.: Brit. Med. J. 2:914.

[19] Eastman, N. J.: Obst. & Gynec. Survey 1:3, 1946.

[20] White, P.: J. Clin. Endocrinol. 3:500, 1943.

[21] Given, W. P., Douglas, R. G., Tolstoi, E.: Am. J. Obst. Gynec. 59:729, 1950.

[22] Reid, E.: Lancet 2:833, 1955.

[23] Jackson, W. P. U.: Brit. Med. J. 2:690, 1952.

[24] Hoet, J. P.: Diabetes 3:1, 1954.

[25] Nelson, H. B., Gillespie, L., and White, P.: Obst. and Gynec. 1:219, 1953.

[26] McKay, D. G., Benirschke, K., and Curtis, G. W.: Obst. and Gynec. 2:133, 1953.

[27] Miller, H. C., and Wilson, H. M.: J. Pediat. 23:3, p. 251, 1943.

[28] Helwig, E. B.: Arch. Int. Med. 65:221, 1940.

[29] Warren, S., and LeCompte, P. M.: The Pathology of Diabetes. Philadelphia, Lea & Febiger, 1952.

[30] White, P., Kosby, P., and Drickers, J.: Medical Clinics of North America 37:1481, 1953.

[31] Potter, E. L., Seckel, H. P. G., and Stryker, W. A.: Arch. of Path. 31:467, 1941.

[32] Miller, H. C.: New Eng. J. of Med. 233:13, p. 376, 1945.

[33] Gilbert, J. A. L., and Dunlop, D. M.: British Med. J. 1: 48, 1949.

[34] Allen, E.: Am. J. Obst. Gynec. 38:982, 1939.

[35] Kriss, J. P., and Futcher, P. H.: J. Clin. Endocrinol. 8:380.

[36] Gilbert, J. A. L.: British Med. J. 1:782, 1949.

[37] Watts, R. M.: Am. J. Obst. Gynec. 30:174, 1935.

[38] Chutkow, J., York, J., Plotz, E. J., and Davis, M. E.: Unpublished data, 1955.

[39] Davis, M. E., and Plotz, E. J.: Endocrinology 54:384, 1954.

[40] Barr, L., Plotz, E. J., and Davis, M. E.: Unpublished data.

[41] Bennett, L. L., and Koneff, A. A.: Anat. Record 96:1.

[42] Appelgarth, A.: Endocrinology 44:197, 1949.

[43] Eranko, O.: Acta Endocrinol. 6:97, 1951.

[44] Field, J. B.: Endocrinology 56:499, 1955.