Acetyl choline Hydrobromide was used in concentration of 1:25 molal. The dosage used was 1 cc. per kilo. All experiments were performed upon normal-fed rats. The 0.2 cc. Somogyi[4] modification of the Shaffer-Hartmann blood sugar method was used. Approximately 50 observations were made upon each point of the curve with the same number in the control series. As can be seen from the accompanying chart, there is a sharp fall in the blood sugar level, the greatest change occurring at the 20-minute interval. The blood sugar has not returned to the original level in 2½ hours.

From these results it appears that the vagus may carry motor impulses to the Islets of Langerhans—if this fall in blood sugar is produced by a liberation of insulin. However, there may be some other mechanism involved, such as the inhibition of glycogenolysis. At the present time, we are unable to explain the mechanism involved in this fall in the blood sugar level. Further work is being done on the subject.

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Further Observations on the Poliocidal Property of Pregnant Mare Serum.*

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We have previously been interested in the changes that occur with pregnancy in man and animals with reference to the ability of the serum and of other biological substances to neutralize the virus of poliomyelitis *in vitro*.[1, 2] In an earlier paper[2] it was reported that the serum of 2 pregnant mares when combined in high dilutions with this virus was capable of bringing about its inactivation. No data were available at that time to indicate whether the normal serum of the same non-pregnant animal possessed similar properties. Again, since our work included tests on 2 animals only, it was unknown how regularly the same phenomenon could be elicited with the serum of other pregnant mares. The most important gap in the observed

4 Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

* Under a grant from the Rockefeller Foundation.

1 Jungeblut, C. W., and Engle, E. T., *J. Immunol.*, 1933, **24**, 267.

2 Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, **27**, 43.

TABLE I.
Virucidal Tests and Gonadotropic Hormone Determinations with Pregnant and Normal Mare Sera.

Mare No.	Type of serum: Pregnancy	Days Pregnant	Date bled	Hormone test	Virucidal Test (0.8 cc. of serum + 0.2 cc. 10% virus emulsion): Test Monkey No.	Dilution of serum	Result		Control Monkey No.	Complete paralysis days
					J20	Undiluted	No paralysis		J11	6
					M13	1:5	" "		M32	8
15	I	72	May, '33* †	Not done	J24	1:10	" "		J11	6
					J60	1:20	" "		J11	6
					J70	1:40	" "		J69	9
					J66	1:60	Complete "	9 days	J69	9
	Normal	61 days after pregnancy	Mar., '34 †	" "	L69	Undil.	Partial "	12 d.	L67	10
					M77	"	No "		M76	7
				.25 cc. 3+	M72	1:10	" "		M76	7
15	II	48	June, '34	.1 3+ 3+	M86	1:20	" "		M79	8
				.05 2+	M87	1:40	" "		M79	8
					N15	1:60	Partial "	9 d.	N25	11
				.25 2+	N7	Undil.	No "		N3	9
	II	132	Sept. '34	.1 1+ 2+	N38	1:20	" "		N25	11
				.05 —	N18	1:40	" "		N25	11
					N70	1:60	Partial "	10 d.	N53	7
				.25 1+	N44	Undil.	Complete "	10 d.	N26	7
	II	198	Nov., 34	.1 ± 1+	N68	"	" "	9 d.	N53	7
				.05 —						
	Normal	9 days after pregnancy	Apr., '35	.25 — .1 — .05 — 0	Q17	"	No "		Q13	8
	Normal	56 days after pregnancy	June, '35	Not done	Q59	"	" "		Q84	9
					Q89	1:5	Complete "	9 d.	Q90	9
					J71	1:20	No "		J69	9
22	I	96	June, '33 *	" "	J72	1:40	" "		J69	9
					J73	1:60	Complete "	11 d.	J69	9

	Normal	4 days after pregnancy	Mar., '34	"	"		L68	Undil.	"	"	9 d.	L67	10
22	II	57	Sept., '34	.25 cc. 4+ .1 4+ .05 4+	4+		N17 N46	" 1:5	No Complete	" "	11 d.	N25 N26	11 7
	II	140 (horse lost fetus)	Dec., '34	.25 — .1 — .05 —	0		N64	Undil.	"	"	6 d.	N53	7
27	I	71	June, '34	.25 2+ .1 — .05 —	1+		M73 M74	" 1:10	" "	" "	8 d. 11 d.	M76 M76	7 7
	I	155	Sept., 34	.25 1+ .1 — .05 —	1+		N8 N19 N20	Undil. " 1:5	Partial Complete "	" " "	14 d. 17 d. 11 d.	N39 N25 N25	9 11 11
	I	226	Dec., '34	.25 ± .1 — .05 —	0		N445 N59	Undil. 1:5	No Complete	" "	8 d.	N26 N53	7 7
	Normal	29 days after pregnancy	Apr., '35	.25 — .1 — .05 —	0		Q16	Undil.	"	"	8 d.	Q13	8
20	I	64	Aug. '34	.25 4+ .1 4+ .05 4+	4+		N10 N47	" "	" Partial	" "	7 d. 6 d.	N3 N26	9 7
	I	183	Dec., '34	.25 — .1 — .05 —	0		N63	"	Complete	"	7 d.	N53	7
8	I	65	Sept., '34	.25 — .1 — .05 —	0		N9	"	"	"	8 d.	N3	9
	I	293	Apr., '35	.25 ± .1 — .05 —	0		Q15	"	"	"	8 d.	Q13	8

* These data have been previously reported in the *J. Immun.*, 1934, **27**, 43.

† These 2 sera have been kindly examined in parallel tests by Dr. N. P. Hudson of the University of Chicago. In agreement with our own results he reported the serum of the pregnant animal as neutralizing and the normal serum as non-neutralizing.

facts, perhaps, was the absence of information as to whether or not the poliocidal power ran parallel with the content of the serum of gonadotropic hormone. It is the purpose of this communication to supply the experimental facts which will answer the questions raised above.

Serum obtained from 5 mares at various stages of pregnancy and, in most cases, after foaling also, were available through the courtesy of Dr. Fred F. McKenzie of the Agricultural College of the University of Missouri, Columbia, Mo. We should like to express our sincere gratitude to Dr. McKenzie for supplying us with this material, for without his untiring cooperation these studies would have been impossible. The samples represented unpreserved, fresh bleedings which were shipped by express. Immediately upon arrival at this laboratory they were tested for virucidal property and for hormone content. The technique of the virucidal test followed the same general principles previously employed in work of this kind, *i. e.*, 0.8 cc. of serum or serum dilution were combined with 0.2 cc. of a 10% virus suspension; the mixtures were incubated for 1½ hours at 37°C., kept in the icebox over night, and the following morning injected intracerebrally into individual monkeys. The animals were carefully observed for one month and absence or presence of paralysis was noted. Control monkeys receiving the same dose of virus mixed with saline accompanied each test. The test for gonadotropic hormone was carried out by injecting duplicate female immature mice with 3 doses of the serum, *i. e.*, 0.25 cc., 0.1 cc., and 0.05 cc., daily for a period of 3 days and examining the animals on the fourth day for evidence of uterine and ovarian stimulation. In reading these tests we were kindly assisted by Dr. E. T. Engle and Miss Parsons.

A total of 19 samples were tested for poliocidal properties, mostly in quantitative titrations, and an almost equal number of parallel hormone determinations were carried out. The data are brought together in Table I.

In analyzing the contents of this table certain conclusions suggest themselves. First, it appears that poliocidal substances are not present in the serum of all mares during pregnancy at the intervals tested in our experiments. Thus, of 5 pregnant mares such substances could be demonstrated, at some time or another, in only 3 animals, 2 failing to exhibit this property on repeated tests. Second, there is no evidence of any clear-cut relation between the poliocidal property of the serum and its content of gonadotropic hormone. While it is true that in some cases poliocidal sera also showed a high hor-

mone concentration and sera with low or no hormone content possessed no poliocidal properties, in other instances sera which gave a high hormone titer failed to neutralize the virus. Third, our data although spreading over several months of pregnancy are insufficient to indicate clearly whether the concentration of poliocidal substances is highest at any given point of gestation. From the data on hand it would seem that there is considerable individual variation between different horses. Fourth, the experimental evidence shows unmistakably that the power of the serum to inactivate the virus is dependent upon changes that occur with pregnancy. Thus, in 2 cases when the normal serum was completely devoid of poliocidal principles, such substances appeared in small amounts coincidental with pregnancy. In a third case, poliocidal substances were not found in the normal serum taken before pregnancy but were apparently present in appreciable amounts in serum drawn after termination of pregnancy. In this latter instance, titrations of the serum during pregnancy indicated exceptionally high concentrations. Of particular interest in this connection are the 2 horses, No. 15 and No. 22, both of which were observed during 2 successive pregnancies. While mare No. 15 went twice through a normal course of pregnancy yielding sera which reacted completely alike, mare No. 22 lost its foal during the latter course of the second pregnancy. With resorption of the fetus both the poliocidal property and the hormone titer disappeared simultaneously.

In summarizing, we should like to emphasize the following points: The presence of poliocidal substances in the serum of pregnant mares can be definitely traced to the immediate effects of pregnancy. This property is present in some and absent in other pregnant mares. It seems to vary with individual horses irrespective of the gonadotropic hormone titer of the serum. When present during pregnancy, this property bears no clear relation to the concentration of gonadotropic hormone in the serum. Further work will perhaps show whether the accumulation of poliocidal substances can be linked more definitely with some other constituent of the serum which increases with pregnancy.