IDENTIFICATION OF ACTH IN HUMAN PLACENTAL TISSUE*

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Studies from the laboratory have revealed the presence of ACTH activity in extracts of pregnancy urine which had been heat-treated to destroy gonadotrophic activity.[7] These observations led to the investigation of placental extracts to ascertain whether the source of the ACTH activity was the placenta rather than the pituitary gland. Certain of the results obtained are reported in this paper.

Although the crude methods of extraction employed do not permit quantitative interpretation of yields, the results of this study indicate that significant quantities of ACTH are produced by the placenta. ACTH activity in extracts from placental tissue has recently been described by Tarrantino[8] and in an isolated study by Jailer and Knowlton.[3]

Identification of the type of activity found in the various fractions of placental extracts is based in this present instance upon previous work from this laboratory.[6] Inhibition of the enzyme, hyaluronidase, by adrenal steroids has been shown to be a normal, physiological mechanism and some of the pharmacological effects observed following the administration of extremely large amounts of adrenal hormones may possibly be related to their influence upon this complex enzyme system. Further, ACTH, presumably through its ability to stimulate the release of effective C-11 steroids, was found to be a potent inhibitor of the hyaluronidase-enhanced spreading phenomenon. And finally, a correlation has been obtained between the ability of the C-11 adrenal steroids to inhibit the hyaluronidase-enhanced spreading phenomenon, and *in vitro* inactivation of hyaluronidase as measured by the inhibition of the release of reducing sugars from hyaluronic acid. These concepts have provided in part the basis for the following experiments.

MATERIALS AND METHODS

Placentas were obtained immediately after delivery.† At first, the placentas were extracted in either of two ways—(i) the placenta was directly frozen in dry ice, stored for one day, and then ground and extracted, or (ii) the still warm placental tissue and blood were ground immediately, and further studies were carried out without intervening lapse of time. The crude dry powders obtained from extraction of frozen placentas were found to possess a markedly lower degree of activity, and much lower yields were obtained from them.

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In part, Lyons' procedure[4] for the extraction of mammotropic hormone from pituitary tissue was employed. While based on speculation, it seemed possible that the major portion of ACTH, if present, would be found in the tissue residue rather than in the original acetone-HCl supernate. Hence, the usually discarded acetone residue of tissue and blood was further examined. In determining the properties of the fractional precipitates in terms of activity and toxicity, as a basis for establishing further experimental procedures, the original extractions were made without reference to quantitative yields, pH, or HCl concentration.

The methods for obtaining Fractions I and II were as follows: Fresh, still warm, whole placental tissue and blood (weighing altogether about 1 kg.) were put through a power grinder within a half hour following delivery, and 4 liters of acetone containing 100 ml. of 35% HCl were added to the semi-solid mass; this was mixed thoroughly for two to four hours and then centrifuged. The supernate was decanted, combined with 5 liters of fresh acetone, and agitated (using a mechanical stirrer) overnight in the cold room. The precipitate was separated by centrifugation, redissolved in a small amount of water, and again precipitated with acetone. This reprecipitation was repeated 4 times, with the final dry yield approximately 15 grams. This precipitate was designated Fraction I.

Because of the high water content of the placental tissue and blood, and the likelihood that the major portion of the ACTH activity might remain in the first acetone residue discarded in obtaining Fraction I, further extraction of this residue was made. The tissue residue remaining after the original acetone extraction was suspended in 1 liter of water, mixed well, and stored in the cold room over night. The solid portion was separated by squeezing the mixture through fine cheese cloth. To the liquid fraction, 4 liters of acetone (no HCl) were added. The precipitate which formed was separated by centrifugation, dissolved in water, and reprecipitated with acetone; this procedure was repeated three times. The final product, when acetone-dried, yielded 25 grams, and was designated Fraction II. By reducing the time of the original acetone extraction of the placental tissue to 15-30 minutes, the weight yield of the final dried Fraction II could be increased considerably with no decrease in activity; for example, average yields of 65 grams of Fraction II and 16 grams of Fraction I could be obtained from individual placentas.

The resulting dry powders were dark to light grey in color, and were very water soluble (example, 10 mg./ml.). These preparations were stored in vacuo. The results of other extraction procedures and further purifications of the extracts will be reported in a subsequent paper.

Fresh beef muscle was ground and extracted in the manner as described for obtaining the Fractions I and II from placental tissue. The beef muscle fractions were combined and their effect on the spreading reaction was determined.

Serum albumin* was used as a further control substance; the final dilutions were made from a sterile solution containing 25 mg./ml.

The test substances, placental fractions I and II, beef muscle extract, and serum albumin, were dissolved in physiological saline, and volumes of 0.2 to 1.0 ml. were injected intraperitoneally. The amounts administered varied from 0.5 to 10.0 mg., and the results were determined after varying time intervals of absorption in normal, adrenalectomized, hypophysectomized, and hypophysectomized-adrenalectomized mice. Equal amounts of saline were injected into control groups of mice, and control studies were routinely made. The experimental animals were set up in groups of equal numbers of males and females. There was no difference with mice of different sex, nor did the volume of diluent used exert any effect on the results.

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* The vial of sterile serum albumin was kindly supplied by Dr. H. B. Vickery of the Connecticut Agricultural Experiment Station.

In other experiments, to determine whether or not a local inhibitory effect could be obtained, amounts of 0.05 to 5.0 mg. of the fractions of placental extract were injected intradermally in normal and adrenalectomized mice, either simultaneously with, or just prior to, the injection of the hyaluronidase and India-ink mixture. The procedures and methods of measurement employed in the hyaluronidase assays have been described in previously published studies.[5, 6]

Male and female mice of the inbred CBA strain,, 10-12 weeks of age and weighing from 25-30 grams were used. Adrenalectomized mice were tested one or six days after adrenalectomy and were not maintained with extra sodium chloride. Hypophysectomized mice of both sexes were used 48 hours after operation; these mice were fed laboratory chow ad libitum and were given 5% glucose in physiological saline to drink. Examination at autopsy showed the completeness of hypophysectomy and only a slight degree of weight loss and adrenal atrophy.

Since the major inhibitory effects have been found on the hyaluronidase-enhanced areas of spreading, this method of reporting results has been used in most of the studies described in this paper. However, in the testing of placental extracts, control injections of saline and India ink alone were compared with the hyaluronidase-enhanced areas of spreading, thus providing a means of semi-quantitive estimation of the inhibition of exogenously added enzyme (see Table 3). The significance of these determinations has been discussed in another publication.[6]

Standardized preparations of testicular hyaluronidase obtained from Dr. Joseph Seifter of Wyeth Inc. were used. Preparations were designated W-108-A, W-109-A, and W-160-A, and varied markedly in degree of spreading activity.

ACTH preparations designated by Armour as H-8412 were used. The contents of one vial was stated to contain an amount equivalent to 35 mg. LA-1-A and to contain 0.2 unit oxytocin and 0.14 unit pressor substance.

The preparations and potency of the human chorionic gonadotrophin and heat-inactivated chorionic gonadotrophin preparations have been described in an earlier publication.[7]

"Adrin," the Sharp and Dohme commercial brand of epinephrine, was used. Final dilutions were made with saline.

Experimental

Normal animals. Table 1, a summary of representative experiments, shows the influence of crude placental extracts on the spreading reaction.

Inhibition of hyaluronidase-enhanced spreading resulted when mice were injected intraperitoneally with amounts of 1 to 10 mg. of the crude placental fractions I and II. Control groups of mice were injected with saline alone. When 1 mg. of Fraction II is injected, a marked inhibition of hyaluronidase-enhanced spreading was observed with roughly a 60% inhibition of added enzyme. When 5 mg. were injected, essentially maximal inhibition of spreading was observed, while complete inhibition of added enzyme was obtained; 10 mg. of Fraction II caused no greater inhibition and produced no toxic effects. With Fraction I, 1 mg. caused a slight but consistent degree of inhibition of spreading, 5 mg. produced an average of 40 per cent inhibition of spreading (72% inhibition of enzyme), while 10 mg. of Fraction I were necessary to obtain maximal inhibition of spreading and complete inhibition of enzyme. Consistently, Fraction II has proved twice as active as Fraction I.

With doses of less than 0.5 mg. of placental fractions I and II, it was impossible to detect hyaluronidase-inhibiting activity; however, in control studies of inhibition it has been consistently seen that large doses of ACTH must also be used, and amounts of ACTH of less than 0.5 mg. were similarly ineffective. Further, administration of 5 mg. of Fraction II produced

TABLE 1

INFLUENCE OF SYSTEMICALLY ADMINISTERED PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE*

I. NORMAL ANIMALS

Substance injected	*Dose** Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*	*% Inhibition of added enzyme†*
				Controls Sq. mm.	*Experimental Sq. mm.*		
Fraction I	1	3	3	534(18)	450(12)	—16	~44
Fraction II	1	1	3	535(12)	355(12)	—34	~62
Fraction II	1	1	24	750(12)	510(12)	—32	
Fraction I	5	3	3	534(18)	315(12)	—40	~72
Fraction II	5	3	3	534(18)	252(12)	—53	~98
Fraction I	10	3	3	623(12)	272(12)	—56	~94
Fraction II	10	3	3	623(12)	250(12)	—60	~99
Fraction II	10	3	3	810*(12)	265(12)	—67	~96
Serum Albumin	1	3	3	540(12)	570(12)	+ 5	
Serum Albumin	5	3	3	540(12)	603(12)	+12	
Serum Albumin	10	3	3	540(12)	630(12)	+17	
Tissue Extract	1	3	3	530(12)	592(12)	+ 1	
Tissue Extract	5	3	3	530(12)	578(12)	+ 8	
Tissue Extract	10	3	3	530(12)	590(12)	+11	

* Enzyme with increased spreading activity used. The increased spreading activity had no effect on the subsequently determined maximal degree of inhibition produced, which represented essentially 100% inhibition of added enzyme.

** Intraperitoneal administration. Diluent = 1.0 ml. physiological saline. Control groups were injected with equal volumes of saline alone.

† See Table 3.

the same maximal degree of inhibition of spreading as was obtained with no less than 3.5 mg. of ACTH from the pituitary.

Administration of 1, 5, and 10 mg. amounts of beef muscle extract or serum albumin was completely without inhibitory effect on the hyaluronidase-enhanced areas of spreading.

While factors of non-specificity or toxicity reactions have been essentially ruled out as an explanation for the observed effects following administration of placental fractions, numerous experiments have also shown that toxic doses of colchicine, spermine, formaldehyde, and certain bacterial agents exert little or no effect in producing inhibition of hyaluronidase-

enhanced spreading. Although the placental extract fractions might conceivably provoke a discharge of endogenous epinephrine, it has been demonstrated that the systemic injection of epinephrine in amounts up to 0.02 mg. per mouse produces little or no inhibitory effect. Only when doses of 0.05 mg. or more per mouse were injected was there any degree of inhibition, and this took place only in animals with intact adrenals (unpublished data).

TABLE 2

INFLUENCE OF PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE

II. ADRENALECTOMIZED ANIMALS

*Substance injected**	*Dose Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*
				Controls Sq. mm.	*Experimental Sq. mm.*	
Fraction I	5	3	1	685(32)	670(6)	−2
Fraction II	5	3	1	685(32)	695(6)	+1
Fraction I	10	3	1	685(32)	850(6)	+19
Fraction II	10	3	1	685(32)	730(6)	+7
Serum albumin	10	3	1	685(32)	678(6)	+1
Beef extract	10	3	1	685(32)	750(6)	+9

* Injected intraperitoneally in volume of 1.0 ml. physiological saline. Control groups were injected with equal volume of saline alone.

NOTE: In intradermal experiments in normal and adrenalectomized animals, these compounds were similarly without inhibitory effect in dosages of 0.05 to 5.0 mg. injected in the intradermal site of hyaluronidase injection, when injections of test substances were made simultaneously with, or just prior to the injection of hyaluronidase and India ink.

The results shown in Table 1 and Figure 1 are representative of several series of experiments in which varying amounts and preparations from different placentas were administrated.

Adrenalectomized animals. Investigations were undertaken to determine the site of action of the placental extract fractions, and whether or not their action might be related to the presence of measurable quantities of the C-11 oxygenated adrenal steroids which are also effective in producing hyaluronidase inhibition.

Groups of male and female mice were tested 24 hours after adrenalectomy. Placental extract fractions I and II dissolved in saline were administered intraperitoneally, and, following arbitrarily selected time intervals of one to six hours, experimental testing with hyaluronidase and India ink solutions was carried out. Table 2 shows that the administration of 1 to 10 mg.

of fractions I or II* was completely without inhibitory effect on the hyaluronidase-enhanced areas of spreading. Serum albumin or beef muscle extract control studies similarly showed no inhibitory effect. In the adrenalectomized animal, cortisone in doses of 0.5 mg. was found effective in producing a marked degree of hyaluronidase inhibition at short post-absorptive time intervals, and Compound F in similar dosages at the longer absorption time intervals was also effective. This would suggest that the activity of these placental extracts was not due to the presence in them of any significant quantities of cortisone or Compound F.

Intradermal experiments in normal and adrenalectomized animals. Intradermal injection of the various placental extract fractions was made in the same site as the injection of hyaluronidase and India ink mixture. The placental fractions were dissolved in saline and combined with the hyaluronidase and India ink solution, or injected just prior to the administration of the enzyme-India ink mixture. The amounts of placental extract injected ranged from 0.05 to 5.0 mg., under the various experimental conditions, and the resulting areas of spreading were measured after time intervals of one to sixteen hours. There was no evidence of inhibition of hyaluronidase-enhanced spreading. Since it has been shown that 0.05 mg. of Compounds E or F, or 0.025 ml. of ACE administered intradermally will markedly inhibit hyaluronidase,[6] the lack of effect produced by large amounts of placental fractions administered intradermally would again indicate the absence of appreciable amounts of the C-11 steroids in the fractions tested.

Hypophysectomized animals. Critical evaluation of the action of these placental extract fractions was obtained by noting their effect on the spreading reaction in hypophysectomized animals. The effects produced by the injection of 1 to 10 mg. of various placental fractions in 48-hour hypophysectomized mice are shown in Table 3 and graphically illustrated in Figure 2. Again, the greater activity of Fraction II is demonstrated. Five mg. of Fraction II or 10 mg. of Fraction I or II produced essentially maximal inhibition of hyaluronidase spreading, with complete inhibition of added enzyme. Inhibition of hyaluronidase did not occur when 1 to 10 mg. of serum albumin or beef muscle extract were administered to groups of hypophysectomized animals.

Potentiation of the hyaluronidase-enhanced spreading appeared to be greater in hypophysectomized animals than was the case with normal animals. It is interesting that hypophysectomized animals better tolerated stress and large doses of these test substances than did adrenalectomized animals under identical experimental conditions. For example, hypo-

* Five and 10 mg. amounts of Fractions I and II were not toxic to the 24-hour adrenalectomized animal; however, the 10 mg. doses proved fatal to the 6-day adrenalectomized animal and in comparison, 5 mg. pituitary ACTH were fatal.

physectomized mice, tested 48 or 96 hours after operation, tolerated without toxic effect doses of 10 mg. of placental extracts, whereas the six-day adrenalectomized animal was killed by 5 mg. ACTH or by 10 mg. of the placental fractions.

A group of hypophysectomized-adrenalectomized animals was given 5 mg. amounts of placental fractions I and II, and hyaluronidase testing

TABLE 3

INFLUENCE OF SYSTEMICALLY ADMINISTERED PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE

III. HYPOPHYSECTOMIZED ANIMALS

Substance injected	*Dose* Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*	*% Inhibition of added enzyme***
				Controls Sq. mm.	*Experimental Sq. mm.*		
Fraction I	2.5	3	3	655(18)	422(6)	—36	~55
Fraction II	1	3	3	655(18)	392(6)	—40	~65
Serum albumin	1	3	3	655(18)	657(4)	±	
Fraction I	5	3	3	655(18)	302(6)	—54	~86
Fraction II	5	3	3	655(18)	244(6)	—63	~100
Beef Extract	5	3	3	655(18)	670(4)	+ 1	
Fraction I	10	3	3	655(18)	268(6)	—59	~94
Fraction I	10	3	3	650(12)	275(6)	—58	~94
Fraction II	10	3	3	650(12)	270(6)	—58	~94
Serum albumin	10	3	3	650(12)	630(6)	— 3	
Beef Extract	10	3	3	650(12)	680(6)	+ 5	

* Intraperitoneal injection in volume of 1.0 ml. saline. Control groups received equal volumes of saline alone.

** Method of calculation:

Example: Control injections of saline and India ink, average area of spreading (24) = 245 sq. mm.

655 — 245 = 410. Spreading in sq. mm. due to hyaluronidase before testing.
268 — 245 = 23. Spreading in sq. mm. due to hyaluronidase after testing.

Per cent inhibition of added enzyme = 410 — 23 = 387; $\frac{387}{410} \times 100 = 94\%$.

carried out as above described. As in the case of the adrenalectomized animals, no inhibition of hyaluronidase-enhanced spreading was produced.

Although the placental extracts were extremely crude, it is interesting that the maximal degree of inhibition produced with the placental fractions was comparable to that observed with relatively similar amounts of pituitary ACTH. The constant degree of inhibition of spreading in the hypophysectomized animals, with extracts made from different placentas, is to be noted.

Effect on adrenal ascorbic acid. In view of the wide acceptance of adrenal ascorbic acid depletion as an indirect measurement of adrenal activity, the effects of placental extracts on the adrenal ascorbic acid levels of a small number of hypophysectomized rats and mice were determined. In hypophysectomized rats the intravenous injection of 1 and 2 mg./100 gm. of fractions I and II produced an average fall in adrenal ascorbic acid of 20% and 50%, while the injection of 0.1 mg. ACTH was followed by an average fall of 55%. In hypophysectomized mice, 2 mg./100 gm. injected intraperitoneally produced an average fall of 32%. In order to obtain sufficient tissue for analysis, the adrenals of three mice were pooled for each determination of ascorbic acid. With the 2 mg./100 gm. dose, there was an average eosinophil fall of 74% in both hypophysectomized mice and rats.

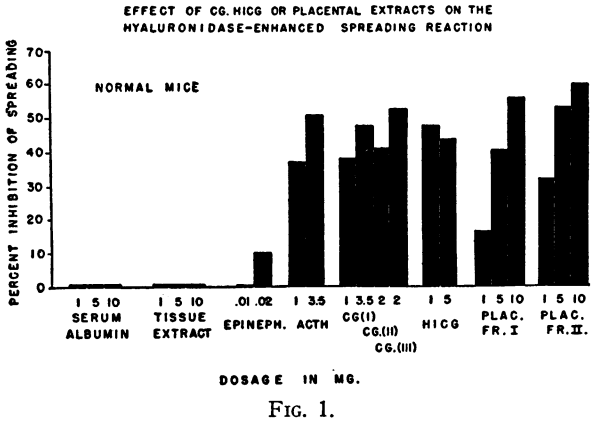

FIG. 1.

DISCUSSION

The markedly similar hyaluronidase-inhibitory effects produced by the various placental extract fractions, by the human chorionic gonadotrophin preparations,[7] compare favorably with the effects produced by the standardized commercial ACTH preparations from the pituitary, in normal, adrenalectomized, and hypophysectomized mice. A graphic summary of some of the results obtained with the use of these preparations in normal animals is seen in Figure 1. Since Fraction II represents material that is normally discarded in the Lyons procedure, it is interesting that Fraction II of the placenta consistently showed a greater degree of activity than did Fraction I, and that more than twice as large a yield of dried powder was obtained in Fraction II. When fresh placental tissue was extracted, the fractions were not only extremely large for the crude procedures employed in extraction, but also possessed ACTH activity in dosage ranges comparable to those for the pituitary preparations. However, placental tissues that had been frozen and stored prior to extraction did not yield fractions comparable in activity or quantity to those obtained with the use of fresh placentas. Thus, a destruction of ACTH activity is indicated, even though the tissue remained in the frozen state. There is indication that this lability of ACTH has been observed in the isolation of ACTH from pituitary tissue by Astwood.[1]

A more critical evaluation of ACTH activity in these placental extracts is seen in Figure 2, which is a graphic summary of results from experi-

ments using hypophysectomized animals. The marked inhibitory effect of the chorionic gonadotrophins and heat-inactivated chorionic gonadotrophin preparations is seen in comparison with results obtained with the crude placental preparations. To indicate further the extent of hyaluronidase inhibition, the results with the placental extract fractions are presented as per cent of actual inhibition of exogenously added hyaluronidase, following the calculation procedure illustrated in Table 3.

The placental tissue fractions caused a marked inhibition of hyaluronidase in both normal and hypophysectomized animals, and with the larger dosages this inhibition approximated 100% inactivation of added enzyme. In all cases the placental extracts were inactive in adrenalectomized animals, and since the inactivation was uniform, this type of experiment was not included in these figures. The possibility that the observed activity was due in any way to the presence of steroids of the nature of cortisone is ruled out by the fact that enzyme inhibition was not observed in adrenalectomized or hypophysectomized-adrenalectomized animals, and by the fact that the placental tissue extracts were without effect when administered intradermally at the site of hyaluronidase injection. Non-specificity was ruled out by the lack of effectiveness of meat extracts and serum albumin, and further supporting this conclusion are the results of numerous studies showing that toxic substances such as formaldehyde, spermine, colchicine, and bacterial agents did not elicit an inhibition of hyaluronidase in the normal or adrenalectomized animal. Epinephrine, in amounts of 0.02 mg. or less per mouse, did not influence the spreading reaction.

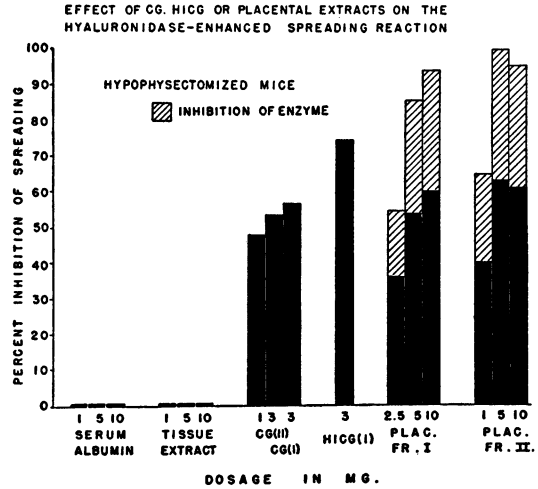

FIG. 2.*

These studies on ACTH activity of placental tissue indicate that the placenta contains relatively large amounts of the hormone. In addition, even though the preparations were extremely crude, there was a striking lack of toxicity with their administration. Since the material is of human

* The more marked inhibitory effect noted with the use of the heat-inactivated chorionic gonadotrophin preparation is due to the fact that a high spreading potency enzyme was employed to test the quantitative aspects of degrees of enzyme inhibition that could be produced. In both types of enzyme testing, essentially 100% inhibition of the exogenously added enzyme was found to have taken place.

origin and presumably would not cause a foreign protein reaction when administered to humans, it is possible that placental ACTH would have an important practical value as there have been recent reports by Feinberg[3] and Thorn[9] of sensitivity to pituitary ACTH preparations. The observation that ACTH activity is obtained with placental extracts is in agreement with the previous report that human pregnancy urine preparations, heated to destroy gonadotrophic activity, possess ACTH activity.[7] These gonadotrophins excreted in the urine during pregnancy have their origin in the placenta,[10] and it is probable that the placental extracts contained the precursor of these excretory products.*

The technique of measuring hyaluronidase inhibition has been developed to the point where it can be applied on a semi-quantitative basis, and has been used as the principal type of evidence for the measurement of ACTH and adrenal steroid activity in this series of studies. However, the pertinent points involved in the establishment of ACTH activity in these placental extract fractions by measurement of hyaluronidase inhibition in the normal, adrenalectomized, or hypophysectomized mouse have been substantiated by studies on adrenal ascorbic acid depletion. In the studies by Tarrantino[8] and by Jailer and Knowlton,[5] depletion of adrenal ascorbic acid has been used as the criterion of ACTH potency. On the basis of these assays, it would appear that extremely large amounts must be administered in order to obtain a significant decrease in adrenal ascorbic acid; however, the gross impurities of the crude extracts must be considered. At the same time, it has been noted that large doses of chorionic gonadotrophins also have to be used in order to obtain adrenal ascorbic acid decreases that are significant. On the other hand, when inhibition of hyaluronidase-enhanced spreading in the hypophysectomized mice is used as a criterion of ACTH activity, the preparations just referred to appear to possess roughly the same order of activity as pituitary ACTH, Armour LA-1-A.

Inasmuch as the experiments herein presented definitely show that there is an adrenal trophic effect, and since epinephrine has been excluded from consideration as a mediator of this action, one must conclude that the effect in all probability is trophic for the adrenal cortex. It is therefore quite appropriate to speak of placental ACTH as a substance which causes the adrenal to produce a factor or factors which inhibit hyaluronidase, but it is not necessary to assume that this placental ACTH has the same physiological characteristics as does pituitary ACTH.

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* Urinary excretion of material possessing ACTH activity is not restricted to pregnancy, since the substance, urogastrone, which possesses many of the chemical properties of chorionic gonadotrophin but which does not have gonadotrophic activity, isolated from normal human male urine, also causes an inhibition of hyaluronidase (unpublished data). It must be remembered that the quantity of material identified as urogastrone in male urine is extremely small, and considerably increases in urine of pregnancy. It is quite possible that the urogastrone isolated from normal male urine originates in the anterior pituitary.

It is obvious that the observations recorded in this paper pose a multitude of questions that must be answered before the relationship between placental and pituitary ACTH can be understood.

Summary

Experimental evidence indicates the presence of ACTH in varying fractions of placental tissue and shows that it is produced in large quantities by this organ. The Lyons' procedure for extraction of ACTH from pituitary tissue is not applicable to the extraction of ACTH from placental tissue and blood.

Administration of various fractions of crude placental extracts to normal or hypophysectomized mice produces a marked inhibition of hyaluronidase-enhanced spreading with essentially complete inhibition of exogenously added enzyme. These fractions are completely without effect in adrenalectomized mice and are without effect when injected intradermally at the site of the hyaluronidase injection.

The inhibitory effect of placental extracts is not due to the presence in them of measurable quantities of steroids of the adrenal cortical type (cortisone or Compound F).

There is a similarity between the inhibitory effects produced by the placental fractions and the results following administration of pituitary ACTH, chorionic gonadotrophins, and heat-inactivated chorionic gonadotrophins.

The presence of ACTH activity in placental extracts is also shown by the depletion of adrenal ascorbic acid and a fall in circulating eosinophils that occurs after their injection into hypophysectomized rats and mice.

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