whether tranquillizers or antidepressants exert an effect on the coumarin response which might correlate with the nature of their central effects.

Guinea-pigs were pretreated with representative tranquillizers and an antidepressant for 4 days, after which their prothrombin response to a standard dose of acenocoumarin[4] was determined. In each instance, the dose of centrally active drug used was approximately the highest tolerated for four daily administrations. Acenocoumarin 4·5 mg was injected intraperitoneally on day 3 and day 4. All animals were killed 24 h after the last dose of acenocoumarin, and cardiac blood was obtained for prothrombin estimation as previously described[4]. Table 1 lists the compounds investigated and the results noted.

The values obtained with saline controls and barbital pretreatment are consistent with previously published results[1] indicating that barbital suppresses the prothrombin response to acenocoumarin; however, neither reserpine nor chlorpromazine had a similar effect. In fact, the data suggest that these tranquillizers exaggerate rather than suppress the prothrombin response.

Table 1

Compound	Dose mg/kg	No. of animals	Prothrombin Average*	time (sec) Range
Saline	—	9	86 (0)	64–118
Barbital	140	4	42 (0)	39–47
Chlorpromazine	2·5–5·0	7	126 (1)	73–180 +
Reserpine	0·5–1·0	8	138 (4)	67–180 +
Desipramine	5–10	8	92 (0)	40–171

* Values over 180 sec were taken as 180 sec in calculating averages. The number of animals with values over 180 sec is recorded in parentheses.

Table 2. PLASMA ACENOCOUMARIN CONCENTRATIONS (mg/l.) IN GUINEA-PIGS 1·5 H AFTER A SINGLE INTRAPERITONEAL DOSE (4·5 MG) WITH AND WITHOUT PRETREATMENT

Pretreatment	No. of animals	Plasma level (mg/l. at 1·5 h) Range	Average
None	5	16–33	20·6
Reserpine (0·5 mg/day intraperitoneally for 3 days)	6	12–34	22·1
Chlorpromazine (2·5 mg/day intraperitoneally for 3 days)	7	11–26	17·3

The apparent exaggeration of prothrombin response to acenocoumarin might be related to higher drug blood levels reflecting inhibition of acenocoumarin metabolism. To test this possibility, the plasma acenocoumarin level was investigated in guinea-pigs following a single intraperitoneal dose (4·5 mg) with and without tranquillizer pretreatment. Previous investigations[1] have shown that pretreatment may alter the plasma acenocoumarin level 1·5 h after dosage. In that investigation pretreatment with barbiturates resulted in levels at 1·5 h which ranged from 2 to 12 mg/l. (nine animals), compared with 16–51 mg/l. (eleven animals) in control animals without pretreatment. In the present investigation (Table 2) similar levels were found in the control group, and the levels in animals treated with reserpine and chlorpromazine were not significantly different from those in the controls. Thus the exaggerated prothrombin response following pretreatment with the centrally active drugs (Table 1) is not explained by a significant change in drug metabolism as reflected by the acenocoumarin plasma level 1·5 h after dosage (Table 2).

Our present work confirms previous reports that barbital pretreatment reduces plasma acenocoumarin levels and prothrombin response in the guinea-pig. On the other hand, pretreatment with the tranquillizers chlorpromazine and reserpine results in an exaggerated prothrombin response without a significant change in plasma acenocoumarin levels. The antidepressant, desipramine, has no influence on prothrombin response.

It may be significant that the tranquillizers, which caused an exaggerated response to acenocoumarin, also enhance NAD synthesis in response to nicotinic acid[5], while barbiturates and the antidepressant enhance neither sensitivity to acenocoumarin nor NAD response to nicotinic acid.

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MURRAY WEINER

New York University Research Service,
The Goldwater Memorial Hospital,
Welfare Island, New York.

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IMMUNOLOGY

Maternal Origin of the Group Specific (Gc) Proteins in Amniotic Fluid

THE origin of the amniotic fluid proteins is at present a source of controversy. Existing information about these proteins, which has been derived mainly from paper electrophoresis[1–6], immunoelectrophoresis[7,8] and immunodiffusion[9], has divided investigators into those who believe that the amniotic fluid proteins arise from foetal serum and those who consider that their origin is maternal. A recent article[10] has shown, by a combination of acrylamide disc electrophoresis and immunodiffusion, that the proteins of the amniotic fluid originate at least partly from maternal serum. It was found that haptoglobin 1–1 was present in all amniotic fluids derived from mothers of this type, while it was absent in the corresponding cord sera. Immunoglobulin A was also present only in amniotic fluids and corresponding maternal sera and absent in cord sera. In addition, transferrin and ceruloplasmin values approximated those of the maternal rather than those of foetal sera.

One of the interesting protein groups observed in amniotic fluids is the group specific (Gc) proteins which are genetically determined. In 1959 Hirschfeld[11] demonstrated the reproducibility of the immunoelectrophoretic patterns of the Gc group. Three distinct phenotypes were found in normal human sera. In a fashion analogous to the haptoglobins, the notations Gc 1–1, Gc 2–1 and Gc 2–2 were adopted.

The present communication reports the Gc patterns in twelve sets of maternal sera, foetal sera and amniotic fluids as determined by immunoelectrophoresis. The procedures and materials used have been described previously[10] except for anti-human Gc-serum (obtained from Lloyd, Inc., Cincinnati, Ohio) which was prepared in rabbits. Our work[10] has shown that the two proteins, haptoglobin 1–1 and IgA, which were present in amniotic fluid and maternal sera, were absent in foetal sera. It was not shown in this preliminary study that any protein present in cord sera was absent in maternal sera and amniotic fluid. We have used the Gc group for such a demonstration. Where mother and foetus belong to a different Gc type, the type of the

Table 1. COMPARISON OF GC PROTEIN PATTERNS IN MATERNAL SERA, AMNIOTIC FLUIDS AND FOETAL SERA

Gc typing set No.	Maternal sera	Amniotic fluid	Foetal sera
1	2–1	2–1	2–2
2	2–1	2–1	2–2
3	2–1	2–1	1–1
4	2–1	2–1	1–1
5	1–1	1–1	2–1
6	1–1	1–1	2–2
7	2–1	2–1	2–1
8	2–1	2–1	2–1
9	2–1	2–1	2–1
10	1–1	1–1	1–1
11	1–1	1–1	1–1
12	1–1	1–1	1–1

Amniotic fluid was obtained by needle aspiration at the beginning of labour. Before use, it was concentrated thirty times by dialysis against solid sucrose. Maternal serum was drawn within 24 h after delivery and cord (foetal) serum was obtained immediately after birth. The twelve sets studied have been grouped according to typing (1–6 and 7–12) rather than the order of testing.

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amniotic fluid should show the source of this protein in the fluid.

Table 1 records our results. It is apparent that where the maternal and foetal Gc patterns differed (sets 1–6), the amniotic fluid pattern and the maternal serum pattern were the same. These findings, together with our previous work, strongly suggest that all proteins in the amniotic fluid are of maternal origin.

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M. USATEGUI-GOMEZ
D. F. MORGAN

Putnam Memorial Hospital Institute
for Medical Research,
Bennington, Vermont.

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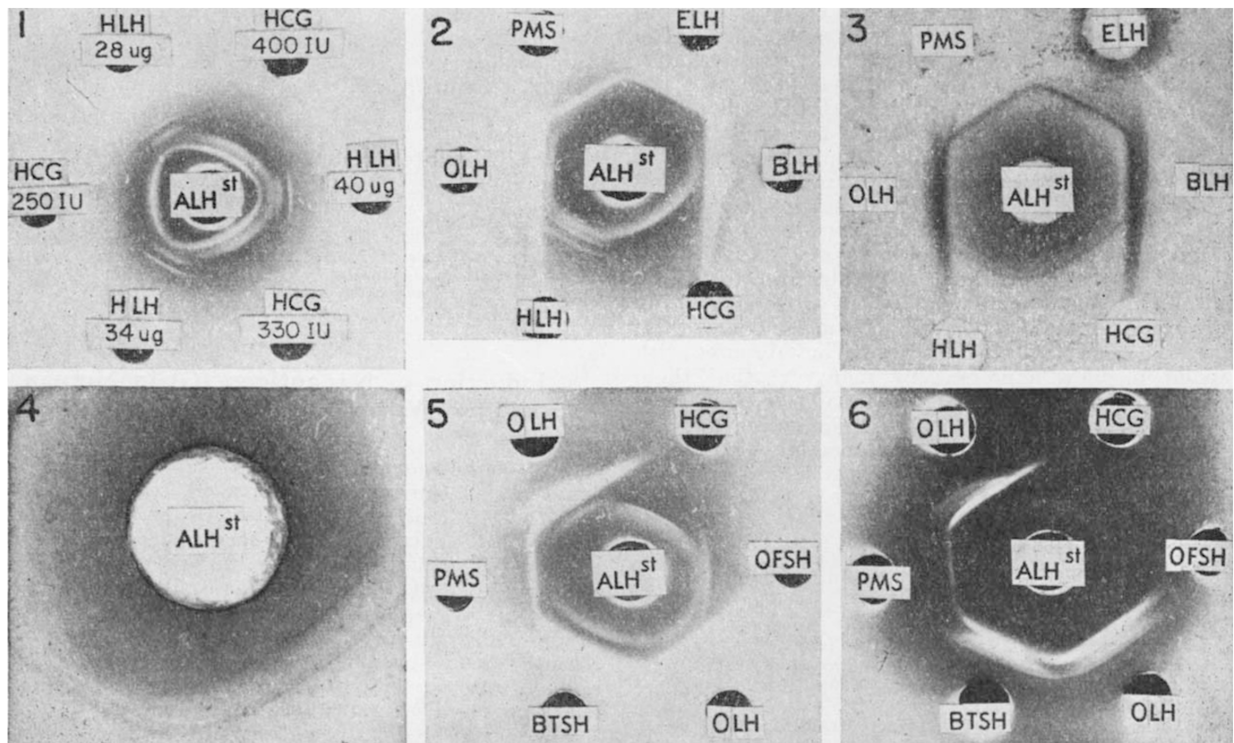
Relationship between Human Luteinizing Hormone and Human Chorionic Gonadotropin

PREVIOUS analysis of a rabbit hyperimmune serum to sheep luteinizing hormone (LH) showed seven components which cross-reacted with sheep LH and follicle stimulating hormone (FSH), bovine thyroid stimulating hormone (TSH), as well as pregnant mare's serum (PMS) and human chorionic gonadotropin (HCG)[1]. The hormone was injected subcutaneously in Freund's complete adjuvant in weekly doses of 1 mg for 6 weeks, and, after a 10 mg booster dose in the seventh week, the animal was rested and bled for the first time in the tenth week. Thereafter, with insignificant variations, the animal was given 1 mg of hormone every other week and bled in the weekly interval between injections. Certain samples from between the twenty-eighth and fifty-second bleedings were pooled and used in the experiments. At the beginning of this period a total dose of 51 mg LH had been given over a period of 14 months. This communication is concerned especially with the cross-reaction of this antiserum with HCG and HLH and the relationship which this shows between the two hormone preparations.

The lines pertinent to this study, those opposite HLH and HCG, were strengthened using a technique similar to continuous antiserum feeding, as in the agar gel double diffusion plate (Fig. 1), compared with Figs. 2, 5 and 6, which were not prepared by this technique. In Figs. 2, 3, 5 and 6, where antiserum to sheep LH absorbed with sheep tissues (ALH^{st}) is reacted with homologous hormone (OLH), some of the seven precipitin lines (resulting from the seven components mentioned already) can be seen, but no attempt was made to delineate and clarify these lines as in the previous experiments. It should be noted that the anti-sheep LH serum was absorbed with sheep tissues under carefully controlled conditions and the completeness of the absorption afterwards tested by several procedures. Thus the possibility of cross-reaction between non-hormonal human and sheep proteins was eliminated.

Fig. 1 clearly shows three common antigens (three precipitin bands) shared by HLH and HCG and a fourth line, the second from the HLH well, which does not appear opposite the HCG well. The slender line nearest the HCG well is remarkable in that it maintained this characteristic appearance under varied experimental conditions. It can also be seen in Figs. 2–5.

Shortening the exposure time of photographs resulted in better definition of the slender line referred to here



Figs. 1–6. Agar double diffusion studies of antigenic relationships between HLH and HCG. Figs. 3 and 4 are stained; Fig. 4 is magnified twice. The "slender" line(s) referred to in the text have been retouched in Figs. 1, 2, 4, and 5. HLH, human LH; OLH, ovine LH; BLH, bovine LH; ELH, equine LH; BTSH, bovine TSH; OFSH, ovine FSH; ALH^{st}, anti-OLH serum absorbed with sheep tissues. Note: the interactions between anti-LH and PMS, ELH, BLH, and BTSH are not relevant to this study.

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