phrine produce arteriolar constriction, increased venous pressure, and diminished splenic weight. Endotoxin, unlike catecholamines, evoked only arteriolar constriction. Hence, the initial transitory resistance increase was probably produced by a direct effect of endotoxin on the splenic arteriolar bed. A local release of some heretofore undescribed arteriolar constrictor is also possible.

The similar response of the splenic veins and the veins of the liver and stomach indicates that, in the portal circulation, persistent venoconstriction is unique to the intestine. Like the liver(2,7) and stomach(5) splenic venoconstriction occurs during the first 5 to 10 minutes, then returns to pre-endotoxin levels.

Previous studies, using phentolamine, have suggested that the total resistance increase may be explained by a systemic release of catecholamines(5,6). Unexplained, however, is the normal splenic venous pressure after 10 minutes coincident with arteriolar constriction. If a catecholamine release were the sole factor responsible for resistance increase, then venous and arteriolar pressures would remain elevated over the same time period. Unlike other vascular beds(1,3,5,6) splenic resistance remained elevated over the full 45 minutes without declining in the "late phase —after 15 minutes"(11).

Summary. These results demonstrate that local injection of the endotoxin *S. typhosa* into the splenic vascular bed of the dog, perfused at a constant flow, results in a progressive increase in calculated vascular resistance. The response of the splenic vasculature to lethal doses of endotoxin, furthermore, is unlike that of the intestine and resembles the response of other organs of the portal circulatory bed, the liver and stomach. Where a persistent venular constriction occurs in the intestine, only a transitory venoconstriction occurs in the spleen.

1. Gilbert, R. P., Gordon, P. J., *Clin. Res. Proc.*, 1956, v4, 110.

2. MacLean, L. D., Weil, M. H., Spink, W. W., Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 602.

3. Kuida, H., Hinshaw, L. B., Gilbert, R. P., Visscher, M. B., *Am. J. Physiol.* 1958, v192, 335.

4. Frohlich, E. D., Scott, J. B., Dooley, E. S., *J. Clin. Invest.*, 1962, v41, 147.

5. Jacobson, E. D., Dooley, E. S., Scott, J. B., Frohlich, E. D., *ibid.*, 1963, v42, 391.

6. Gillenwater, J. Y., Dooley, E. S., Frohlich, E. D., *U. S. Army Med. Res. Lab. Rep. No. 551,* 1962.

7. MacLean, L. D., Weil, M. H., *Circulation Res.*, 1956, v4, 546.

8. Hinshaw, L. B., Nelson, D. L., *Am. J. Physiol.*, 1962, v203, 870.

9. Frohlich, E. D., Gillenwater, J. Y., *ibid.*, 1963, v204, 645.

10. Ottis, K., Davis, J. E., Jr., Green, H. D., *ibid.*, 1957, v189, 599.

11. Gilbert, R. P., *Physiol. Rev.*, 1960, v40, 245.

Received May 9, 1963. P.S.E.B.M., 1963, v113.

Serum Proteins and Glycoproteins in 2 Strains of Mice Differing in Teratogenic Potential.* (28426)

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Protein metabolism has been implicated in teratology. Gillman *et al.*(1,2) suggested that altered behavior of the maternal proteins might be correlated with the teratogenic action of trypan blue on rabbit fetuses. Langman(3) found a greater risk of abnormal children in 19 pregnant women without evident disease but with altered serum protein fractions. Experimentally in rabbits, Langman and van Drunen(4) found, following trypan blue, increased α- and β-globulins in serum.

* Aided by grants from U. S. Public Health Service and from Association for the Aid of Crippled Children.

Differing mouse strains (Albino Webster, C_3H, Strong A show constant small differences in blood protein patterns and results in this and other laboratories have shown these to be affected by cortisone(5,6,7). It was further shown by Loevy(8), following earlier work of Fraser(9) and Kalter(10) that 1.25 mg cortisone given to the pregnant mouse for 4 days beginning on the 11th day of pregnancy induced 100% cleft palate in offspring of the Strong A and only 36% in those of the C_3H strain.

The objective of this study was to evaluate possible relations between cleft palate induction by cortisone and changes in protein metabolism of the mother as reflected by serum protein and glycoprotein fractions.

Methods. Strong A and C_3H mice were fed water and Purina Chow *ad libitum*. Fifteen females of each strain were separated and bled by capillary tubes from the retrobulbar plexus of the eye. Usually 6 tubes (1.3 × 75 mm) of blood per animal were collected. After a one-week recovery period, the mice were injected daily for 4 days with 1.25 mg cortisone (cortone acetate, Merck). They were again bled some hours after the last injection. After a further recovery period, the animals were mated by caging overnight with males. When a copulation plug was found, this was counted as day zero of pregnancy. A total of 15 pregnant females of each strain were injected with 1.25 mg cortisone from day 11 to day 14 of pregnancy, inclusive. Blood was taken some hours after the last injection. As controls, 15 uninjected pregnant mice of each strain were bled on day 14 of pregnancy. The capillary tubes were spun in a Spinco centrifuge designed for this purpose, and usually 0.3 ml serum was recovered.

The following analyses were done on each serum sample: total protein, Weichselbaum (11); paper electrophoresis, Spinco, Model R; protein fractions, Jencks' *et al.*(12) method with modifications given in Spinco manual; glycoproteins (PAS positive), Köiv method outlined by Block *et al.*(13). Electrophoretic strips were scanned by the Spinco Analytrol.

Protein fractions were calculated as absolute quantities based on total protein; glycoprotein fractions were recorded as percentages in the sample analyzed.

Results. Results are shown for serum proteins and protein fractions (Table I) and for glycoprotein fractions as stained by periodic-acid leucofuchsin (Table II). Some intergroup comparisons of total protein and protein fractions were made (Table III).

Discussion. Between the 2 strains Strong A and C_3H which are respectively susceptible (100% incidence) and resistant (36% incidence to induction in offspring of cleft palate with cortisone, there are no significant differences in total proteins or protein subfractions, with the exception of γ-globulin which is twice as high in Strong A, but is reduced to one-half by pregnancy. Cortisone treatment and pregnancy *per se* modify the amounts of plasma proteins and subfractions with 2 dominant tendencies which are partly neutralized and partly maintained when cortisone is given to the pregnant animal.

Pregnancy leaves total proteins unchanged, but lowers albumin and elevates α_2-globulins in both strains and also moderately raises β-globulins in C_3H (Table IIIA). The ratio albumin/globulin is therefore much lower in pregnant females.

Cortisone elevates total proteins and α_2-globulins in both strains and increases β-globulins in Strong A and α_1 globulins in C_3H (Table IIIB). Albumin is unchanged, but the net effect is again to lower the albumin/globulin ratio.

When pregnant animals are treated with cortisone (Table IIIC) the tendency for albumins to fall is reversed. The increases in globulin fractions are maintained but not necessarily enhanced, with the exception of a further increase in the α_1-globulin of C_3H. The albumin/globulin ratio is lowered, but not as much as in cortisone injected non-pregnant mice.

The distribution of carbohydrate-containing proteins normally differs little between the 2 strains (Table II) when expressed as percentages of total periodic acid-leucofuchsin staining material. About 10% of this activity migrates with the albumin and γ-globulin fractions. Cortisone and pregnancy

TABLE I. Total Proteins and Protein Fractions Studied by Electrophoresis Before and After Cortisone in Normal and Pregnant Mice.

	Strong A				C_3H			
	Non pregnant		Pregnant		Non pregnant		Pregnant	
	Untreated	After cortisone	Untreated	After cortisone	Untreated	After cortisone	Untreated	After cortisone
Total proteins	5.55 ± .81	7.02 ± 1.02	5.36 ± .55	6.25 ± .97	5.24 ± .79	6.37 ± 1.06	5.34 ± .63	5.98 ± .68
Albumins	3.25 ± .54	3.61 ± .11	2.59 ± .43	3.24 ± .73	3.58 ± .70	4.01 ± .83	2.47 ± .72	3.18 ± .38
α_1	.21 ± .07	.27 ± .07	.24 ± .10	.26 ± .15	.15 ± .05	.36 ± .14	.34 ± .15	.71 ± .21
α_2	.33 ± .09	.88 ± .21	.71 ± .22	.79 ± .28	.37 ± .13	.94 ± .29	.82 ± .25	.64 ± .11
β	1.20 ± .30	1.80 ± .44	1.54 ± .40	1.71 ± .57	.94 ± .19	.90 ± .27	1.45 ± .80	1.17 ± .19
γ	.42 ± .15	.45 ± .40	.26 ± .14	.22 ± .12	.19 ± .08	.20 ± .09	.21 ± .04	.23 ± .05
Alb/glob	1.50	1.06	.94	1.09	2.16	1.50	.87	1.16

TABLE II.* P.A.S. Stained Glycoproteins in Normal and Pregnant Animals Before and After Cortisone.

	Strong A				C_3H			
	Non pregnant		Pregnant		Non pregnant		Pregnant	
	Untreated	After cortisone	Untreated	After cortisone	Untreated	After cortisone	Untreated	After cortisone
Albumin—CHO	2.3 ± .67	1.9 ± .7	3.9 ± .88	2.3 ± .87	4.3 ± 1.3	3.2 ± 1.1	3.1 ± 2.0	3.7 ± 1.5
α_1—CHO	18.6 ± 2.89	8.6 ± 4.07	11.6 ± .88	9.7 ± 6.2	20.7 ± 1.8	12.1 ± 6.5	12.9 ± 3.8	10.9 ± 4.2
α_2—CHO	39.3 ± 5.65	55.9 ± 29.4	40.5 ± 12.4	45.7 ± 9.9	32.5 ± 2.6	55.5 ± 10.7	46.5 ± 8.8	46.9 ± 6.2
β—CHO	34.6 ± 3.8	24.9 ± 5.30	36.0 ± 6.10	37.6 ± 8.4	35.6 ± 2.1	25.6 ± 7.9	33.2 ± 4.6	32.2 ± 3.0
γ—CHO	7.2 ± 3.09	8.8 ± 3.7	7.1 ± 4.5	4.4 ± 2.2	7.1 ± 1.7	3.1 ± .2	4.0 ± 1.7	6.1 ± 1.0

* All values given in percentages.

TABLE III. Summary of Intergroup Comparisons Where Significant Differences in Total Protein and Protein Fractions Were Present Before and After Cortisone Treatment in Non Pregnant and Pregnant Mice (from Table I).

State of animal		Strains: Strong A	Strains: C_3H
A			
	Total proteins	—	—
	Albumins	.05 to .02 (decr)	.01 to .001 (decr)
(1) Non pregnant, untreated	α_1	—	—
(2) Pregnant, untreated	α_2	.05 to .02	.01 to .001
	β	—	.02 to .01
B			
	Total proteins	$<$ .001	.01 to .001
	Albumins	—	—
(1) Non pregnant, untreated	α_1	—	—
(2) " " after cortisone	α_2	.01 to .001	.01 to .001
	β	.02 to .01	—
C			
	Total proteins	.02 to .01	—
	Albumins	.05 to .02	.02 to .01
(1) Pregnant, untreated	α_1	—	.05 to .02
(2) " after cortisone	α_2	—	—
	β	—	—

Figures indicate P values of the difference between first and second state of animals. They represent significant *increases* except in 2 cases noted as decreases.
(—) indicates no significant difference ($P<.05$).

induce rather similar, but not synergistic effects on the remaining major globulin-carbohydrate fractions.

Rather consistent was the lowering in percentage of α_1-globulin by cortisone and by pregnancy in both strains. Cortisone alone lowered the β-globulin. The α_2-globulin fraction was increased by cortisone in both strains, while pregnancy *per se* increased α_2-globulin only in C_3H mice.

On the whole, globulin-carbohydrate fractions in pregnancy were not modified significantly by the addition of cortisone and the effects of cortisone were generally less marked than in non-pregnant animals.

The teratomatous lesions induced by cortisone in Strong A mice have been attributed to alterations of materials in the palatine shelves, including the sulfated acid mucopolysaccharides(14), and substances reacting with the aldehyde fuchsin stain(15). Further studies are necessary to ascertain the precise nature of the tissue elements involved and the locus of cortisone action. The present experiments show that maternal serum proteins, protein fractions, and protein bound carbohydrate fractions are modified by cortisone, by pregnancy and by pregnancy plus cortisone in complex ways. While the 2 strains in fact react differently to cortisone in respect to protein behavior and teratogenic effects, it seems safer to treat these as coincidental actions of cortisone on protein metabolism (or mobilization) and on the tissues related to cleft palate, rather than to infer a direct relation of protein metabolism and teratology.

Summary. In 2 strains of mice, Strong A and C_3H, which are respectively susceptible and resistant to induction of cleft palate in offspring when cortisone is given to pregnant mothers, it was found that cortisone, pregnancy *per se* and pregnancy plus cortisone lead to definite and different effects on serum proteins, protein fractions, and protein-bound carbohydrate fractions. A direct connection between protein metabolism and cleft palate is not inferred from the results.

1. Gillman, J., Gilbert, C., Gillman, T., Spence I., *S. African J. Med. Sci.,* 1948, v13, 47.

2. Gillman, J., Gilbert, C., Spence, I., Gillman, T., *ibid.,* 1951, v16, 125.

3. Langman, J., *Internat. Symposium on Cong. Anomalies of the Face and Assoc. Structures,* 1961, p149.

4. Langman, J., van Drunen, H., *Anat. Rec.,* 1959, v133, 513.

5. Bestetti, A., *Chemical and histochemical studies*

on human and experimental amyloidosis, 1959, M.S. Thesis, Univ. of Illinois, Chicago.

6. Lathvalati, J., *Acta Endocrinol.* (Kbh)., suppl., 1953, 16.

7. Pirani, C. L., Bestetti, A., Mombelloni, P., Catchpole, H. R., *Atti X Congresso Lega Internat. Contro il Reumatismo,* 1961, v2, 912.

8. Loevy, H., *Anat. Rec.,* 1963, v145, 117.

9. Fraser, F. C., *First Internat. Conf. of Cong. Malformations,* 1960, p179.

10. Kalter, H., *Genetics,* 1954, v39, 185.

11. Weichselbaum, T. E., *Am. J. Clin. Path.,* 1946, v16, 40.

12. Jencks, W. P., Jetton, M. R., Durrum, E. L., *Biochem. J.,* 1955, v60, 205.

13. Block, R. J., Durrum, E. L., Zweig, G., *A manual of paper electrophoresis,* Acad. Press, New York, 1955.

14. Walker, B. E., *2nd Annual Meeting of Teratology Soc.,* 1962, p11.

15. Clark, K. H., *Genetics,* 1956, v41, 637.

Received April 4, 1963. P.S.E.B.M., 1963, v113.

Iontophoresis of Potassium for Experimental Determination of Pain Endurance in Man.* (28427)

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The value of experimental work with pain sensation has been a subject of heated debates for many years. Beecher(1) pointed out that the difficulty is largely based on differences between clinical observations and conclusions drawn from laboratory experiments. According to definition pain is a disagreeable sensation. However, the threshold pain used almost exclusively in laboratory experiments can hardly be considered disagreeable. If in daily life we bump an elbow or touch a sharp instrument producing threshold pain, it would probably not even be considered as pain by the subject. Clinical pain has an inherent threat content, which is absent under experimental conditions. Beecher(1) shows that clinical pain varies considerably between subjects, while Hardy(2) in carefully controlled experiments showed that threshold pain is a constant factor. Therefore it is felt that experimental tests of pain endurance may be closer to clinical pain than tests of threshold pain.

Theoretically, a test of pain endurance should comply with the following criteria: 1. The test should be specific for pain sensation. 2. The stimulus should be well controlled and accurately measurable. 3. There should be a direct relation between measured intensity of the stimulus and resulting intensity of pain sensation. 4. The test should be reproducible and variations should represent physiological variability of pain sensation. 5. The test should produce a minimal degree of tissue damage and there should be no danger of severe or lasting injury. 6. The equipment should be simple, rugged and inexpensive.

In earlier work(3) various tests were used for pain endurance. However, none of them are suitable for evaluation of interpersonal differences. On immersion of the hand in ice water, fluctations of pain sensation are observed, rather than a steady increase with time. Therefore, time of immersion is no indication of pain intensity. Using heat as the stimulus, burn injury is produced at 50°C, while the endurance limits are reached only at 60°C. The same danger of injury prevents the use of electrical stimulation of the tooth. In ischemic contraction a blood pressure cuff, inflated to 200 mm Hg, is applied to the upper arm and the rhythmic hand contractions against a fixed resistance are counted. This method works well for evaluation of drug effects where each subject serves as his own control. However, it is not suitable for determination of interpersonal differences, as the threshold varies with muscular development so that the mean endurance limit for females is lower than the threshold for males.

On the basis of previous experience with iontophoresis of potassium(4), this method

* Partially supported by NASA grant.