TABLE I. Degree of Leukocytosis Produced in Mice at 4 Hours by Various Doses and Routes of Administration of LFP. 5 mice in each exp.

Route of administration	Dose LPF (mg/kg)	Avg change in white cell count at 4 hr (% of pre-inj. count)
Intraperitoneal	5	110
	10	119
	50	153
	100	119
	*	96
Intravenous	10	105
	50	156
	*	107

* Saline control.

TABLE II. 30-Day Survival of Irradiated Mice Treated with LPF or Saline Intraperitoneally.

No. mice	Dose of X-rays (roentgens)	Treatment	30-day survival	% survival
10	450	50 mg LPF/kg for 6 days	9	90
	500		4	40
	600		2	20
	700		0	0
30	450	Saline inj. controls	22	73
	500		13	43
	600		2	7
	700		0	0

vival. Analysis of the data by the probit analysis method described by Finney(4) gives an LD_{50}^{30} value of 489 r for the control mice and 518 r for the mice injected with LPF. These differences are not considered significant.

Discussion. Treatment of irradiated mice with leukocytosis-promoting factor did not significantly increase their 30-day survival. This fact strongly suggests that if survival of irradiated mice into which normal spleens have been implanted is due to a "humoral" factor released by the spleen, such a factor is unrelated to the specific leukocyte-promoting factor obtained from sterile exudates.

Summary. The present study does not contribute to the discussion as to whether or not a "humoral" substance is secreted by the spleen. It does show, however, that LPF, a logical therapeutic agent, is ineffective in reducing X-ray mortality in mice.

We are indebted to Dr. Valy Menkin for providing us with a potent sample of LPF and for helpful suggestions as to the plan of the experiment.

1. Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J., and Eldredge, J. H., *J. Lab. and Clin. Med.*, 1951, v37, 683.

2. Menkin, V., *Am. J. Path.*, 1940, v16, 13.

3. ———, *Am. J. Path.*, 1943, v19, 1021.

4. Finney, D. J., *Probit Analysis,* Cambridge Univ. Press, London, 1947.

Received October 27, 1952. P.S.E.B.M., 1952, v81.

Mechanism of Renal Glycosuria in ACTH-Treated Premature Infants.* (19934)

DAVID H. WEINTRAUB, PHILIP L. CALCAGNO, MARY K. KELLEHER, AND MITCHELL I. RUBIN.

From the Statler Research Laboratories, Buffalo Children's Hospital and the Department of Pediatrics, School of Medicine, University of Buffalo.

In a group of 7 premature infants given ACTH in the treatment of early retrolental fibroplasia, 5 developed glycosuria, and in none of these was there an associated hyperglycemia. These data implied a glomerulotubular functional imbalance in the handling of glucose. Dustan *et al.*(1) concluded that glycosuria during cortisone and ACTH administration results from a depression of the glucose Tm. However, Earle *et al.*(2) concluded from their studies that there was no consistent decrease in the maximum ability (Tm) of the tubules to reabsorb glucose under the influence of ACTH and believed that the glycosuria results from a rise in glomerular filtration rate producing an increased glucose

* Aided by a grant from Mead Johnson and Co.

TABLE I. Effect of ACTH on Renal Function.

	Name & date	Age, days	Wt, g	Therapy	GFR, ml/ min./1.73 M²	TmG, mg/ min./1.73 M²	GFR / TmG	S.A.
				Patients with glycosuria				
1.	W 5/13/51	12	1650	Prior to ACTH	43	93	.47	.144
					41	88	.46	
	W 6/14	33	1955	ACTH for 21 days	27	15	1.80	.161
					26	14	1.80	
	W 6/30	49	2240	Post ACTH period	70	177	.40	.177
					70	169	.42	
2.	JL 7/25/52	26	1670	Prior to ACTH	22	48	.46	.145
					25	48	.52	
	JL 8/ 8/51	39	1690	ACTH for 13 days	43	53	.81	.146
					41	55	.75	
3.	JL 7/26	27	2000	Prior to ACTH	43	86	.50	.163
					44	98	.45	
	JL 8/10	42	2080	ACTH for 10 days	43	51	.85	.168
					41	42	.97	
	JL 8/22	54	2160	ACTH for 22 days	67	74	.91	.172
					55	67	.82	
4.	P 6/21	54	1820	ACTH for 17 days	61	92	.67	.154
					59	89	.66	
5.	M 1/30/52	87	2750	ACTH for 21 days	68	92	.74	.202
					64	83	.76	
				Patients without glycosuria				
6.	S 11/ 1/51	37	2000	Prior to ACTH	31	60	.51	.163
					36	68	.53	
	S 11/30	67	2830	ACTH for 21 days	46	89	.52	.206
					41	83	.49	
7.	N 12/ 5	41	2270	Prior to ACTH	34	75	.47	.177
					34	72	.50	
	N 12/12	47	2320	ACTH for 6 days	31	71	.43	.180
					29	75	.49	

load which is above the capacity of the normal tubules to reabsorb. Because of these conflicting views, and in order to determine the mechanism involved in the glycosuria of these premature infants, the following study was undertaken.

Materials and methods. Premature infants weighing between 1650 and 2750 g, and between 12 to 87 days of age were studied. Glomerular filtration rate and glucose Tm were estimated before, during, and after a period of ACTH therapy. The glomerular filtration rate was measured by inulin clearance. The inulin was administered subcutaneously as a 10% solution 90 minutes before estimating the glomerular filtration rate. Inulin was determined by the method of Hubbard and Loomis, using the acid mixture of Harrison(3). To obtain the glucose Tm a 15 to 20% glucose solution was infused intravenously at a rate of about 1 ml/min. (200 mg glucose/min.). Forty-five minutes were allowed for equilibration of the glucose. Capillary blood glucose values of 450 to 1020 mg % were obtained. In each case the load/TmG ratio was over 1.30. Glucose was determined by the method of Nelson(4). Both glomerular filtration rate and glucose Tm were determined within 3 to 4 hours after the last injection of ACTH. ACTH was given intramuscularly in a dosage of 40 mg daily for the first 2 weeks, then reduced to 20 mg daily for the third week, and finally to 15 mg daily for the fourth week. The total daily dosage was divided into 4 equal parts and given at 6-hour intervals. Since varying time elapsed in these patients between the control and the experimental periods, it became important to estimate the normal maturational change in glomerular filtration rate and glucose Tm occur-

TABLE II. Glucose TmG in Premature Infants.

Name & date	Age, days	Wt, g	Therapy	GFR, ml/ min./1.73 M²	TmG, mg/ min./1.73 M²	GFR / TmG	S.A.
8. M 10/ 4/51	27	1930	None	42	72	.59	.160
				48	82	.58	
M 10/17	41	2250	"	54	119	.46	.177
				44	125	.35	
9. E 9/13	30	2290	"	39	85	.46	.179
				36	73	.50	
E 9/20	47	2550	"	46	101	.46	.192
				46	110	.42	
				46	100	.46	
10. St. 7/12	7	1390	"	29	50	.58	.126
				30	47	.63	
11. L. 7/25	26	1670	"	22	48	.46	.145
				25	48	.52	
12. JI. 7/26	27	2000	"	43	86	.50	.163
				44	98	.45	

ring over this period of time. For this reason a group of 5 premature infants were studied to determine the effects of aging alone on these functions over a similar period of time.

Results. Table I shows the data on a group of 5 premature infants who developed glycosuria under ACTH therapy, and 2 who did not develop glycosuria. The effect of ACTH on glomerular and tubular functions in these maturing infants can be gauged only by comparison with the changes in glomerular filtration rate and glucose Tm naturally occurring with aging. Table II shows the effect of aging alone on these two functions.

It is apparent from Table II that during the length of time involved in the ACTH study (13 to 37 days) there is but small increase in glomerular filtration rate, but a somewhat more significant rise in glucose Tm as a result of growth. In the infants receiving ACTH, a rise in glomerular filtration rate over the expected increase occurred in 3 of the 5 infants. In one case it was apparent on the 13th day of ACTH administration; in another it occurred only after more prolonged ACTH effect (27 days), and the rise was slight in the third case after 21 days. In case I there was a fall in glomerular filtration rate. This patient developed an associated albuminuria and hematuria. In the fifth child the glomerular filtration rate was essentially unchanged. In the 5 infants where glucose Tm was compared before and after ACTH, there was a fall in 2, 2 remained unchanged, and in the fifth child there was a rise. The rise in glucose Tm in this latter patient treated for a 30-day period approximately equals the rise found in the 2 normal infants over a shorter period of time as a result of normal growth. In the 2 instances where the glucose Tm was unchanged with ACTH, we would have anticipated over this period of time a rise comparable to the two control cases (25% to 50%).

The imbalance of glomerulo-tubular function for glucose is strikingly demonstrated by comparing the GFR/TmG ratio in the ACTH-treated infants with the preACTH period and the control non-ACTH group. In the non-ACTH infants the ratio varies between 0.35 to 0.59. This is in agreement with values obtained in premature infants by Tudvad(5). This author also demonstrated a fall in this ratio with aging. In the ACTH-treated infants with glycosuria the ratio ranged from 0.66 to 1.80. This rise in ratio cannot be accounted for by increasing maturity. In infants 4 and 5, where no preACTH period was obtained, the high GFR/TmG ratios indicate the ACTH effect.

In 2 infants where glycosuria was not induced by the ACTH the ratio of GFR/TmG was not elevated. There was also no depression of the glucose Tm in these 2 infants. In the case where glomerular filtration rate was increased the glucose Tm was increased pro-

portionately, keeping the GFR/TmG ratio unchanged. The reason for the lack of ACTH effect in these 2 children is not apparent. However, in case 7 the lack of response may be related to the short period of ACTH administration, (6 days).

Conclusions. Glycosuria resulting from ACTH administration in premature infants is due to an imbalance of glomerulotubular function demonstrated by a rise in GFR/TmG ratio. The cause of the rise in this ratio varied and was due either to a disproportionate rise in glomerular filtration rate or fall in glucose Tm. The nonglycosuric ACTH-treated infants did not show this rise in the GFR/TmG ratio.

1. Dustan, H., Corcoran, A. C., Taylor, D., and Page, I. H., *Arch. Int. Med.*, 1951, v87, 627.
2. Earle, D. P., Alexander, J. D., Farber, S. J., and Pellegrino, E. D., *2nd Clin. ACTH Conf.*, 1951, v1, 139.
3. Harrison, H. E., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 111.
4. Nelson, Norton, *J. Biol. Chem.*, 1944, v153, 375.
5. Tudvad, F., *Scandinav. Clin. and Lab. Invest.*, 1949, v1, 281.

Received July 29, 1952. P.S.E.B.M., 1952, v81.

A Glass Wool Matrix for Roller Tube Tissue Cultures.* (19935)

ARTHUR W. FRISCH.

From the Department of Bacteriology and Immunology, University of Oregon Medical School, Portland.

The isolation and identification of the poliomyelitis group of viruses by means of tissue culture has stimulated interest in the application of the roller tube technic to viral and rickettsial agents in general(1-6). The above method involves imbedding fresh tissue in a chicken plasma clot and the cytologic examination of the fibroblast outgrowth at intervals. The use of a clot as a matrix for growing cells has a number of recognized disadvantages among which are listed the following: 1) preparation and preservation of plasma and chick embryo extracts is time-consuming and costly; 2) there is often clouding and liquefaction of the clot; 3) subcultures are tedious and exacting to prepare; 4) staining procedures often necessitate sectioning; 5) the composition of plasma and embryo extract is variable and chemically undefined; 6) plasma may contain contaminating viruses. Some of these objections may be circumvented by means of the glass wool roller tube technic herein described.

Methods. A very thin mat of glass wool† is cut into an approximately 2¼ by ¾ inch piece. This is placed between two metal blocks measuring 1¾ inches long and ¼ inch wide, and the free edges of the fibers are fused with a flame. The glass mat is then washed with ethyl ether and sterilized in the oven at 160°C for 1 hour. Heart or chorioallantoic membrane from a 10-day chick embryo is minced in a petri dish containing 1 part Simms' ox serum ultrafiltrate and 3 parts Hanks' balanced salt solution(5), and a concentration of 100 units of penicillin and 0.1 μg of streptomycin per ml. Three to 6 fragments of thoroughly washed tissue are arranged lengthwise along the inner surface of a pyrex glass tube measuring 16 x 150 mm. The glass wool mat is then placed over the tissue, pressed down gently and the tube cooled for a few seconds in an ice water bath. With a capillary pipette 2-3 drops of sterile, liquid, 2.5% washed agar-agar in saline are placed over the glass mat at the extreme top and bottom. The tube is again immersed in the ice bath in order to solidify the agar. This procedure serves to fix the mat to the sides of the tube and to trap the tissue beneath the glass wool. One

* Aided by a grant from the National Foundation for Infantile Paralysis.

† Pyrex Brand Filtering Fibre; Owen-Corning Fiberglas, Corning, N. Y.,