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FINAL REPORT OF WORK CARRIED OUT UNDER CONTRACT
WITH LOS ALAMOS NATIONAL LABORATORY

Period covered: June 1, 1989 - May 31, 1990

As indicated in the last report, after Ms. Doris Miyashiro resigned as a part-time technician, Mr. Leo Martinez was transferred to her position. When Dr. Kowluru came to this laboratory for further micropuncture studies, I had him start training Mr. Martinez in preparation of micropipettes. Training was later completed by S. Solomon. The reason for this approach was that Dr. Kowluru was better at sharpening pipettes than was Dr. Solomon and it was his position that training of Mr. Martinez would be optimal by following this sequence. One difficulty which was encountered was that the pipettes produced were not clean enough for good microinfusion or perfusion studies and Mr. Martinez has to be given special instruction on preparing clean micropipettes. This procedure involved: 1) soaking glass tubing in sulfuric acid-dichromate solution, 2) washing out this cleaning solution with distilled water; and 3) removing the water by an acetone rinse, and 4) drying. After pipettes are pulled and sharpened, the cleaning procedure is repeated by aspirating each solution into the tip of the pipette. Kowluru then revisited our laboratory in early June (as indicated in the previous report and completed some tubule infusion studies.

At this time it was noticed that the pump we were using had a bent drive wire and solution was being delivered intermittently. When Dr. Kowluru went to India, Dr. Solomon designed a new pumping procedure. The driving system consisted of an adjustable Sage pump adjusted to very low speeds. The solution was contained in a 50 ml. Hamilton syringe which was attached to heavy walled polyethylene tubing. The tubing was flared at the delivery end. The whole system was carefully filled with mineral oil, care being taken to exclude all air bubbles. The delivery

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pipette was filled in part with test solution and the remainder with oil. It was mounted in a Leltz pipette holder with the end cut off. In this way the pipette extended through the holder and it was possible to make a friction grip through the pipette end and the oil filled flared end of the polyethylene tube. In all of these steps it is critical that air be excluded.

The use of the pump system was validated to show 1) test solution was forced out of the pipette tip as soon as the pump was turned on and stopped as soon as the pump was turned off. Failure to meet this criteria indicates either the presence of air bubbles or that the pulled polyethylene tube is too flexible to, 2) Test solution had to be delivered at the same rate, before puncture, during puncture and after puncture. This point was established by following the movement of the syringe plunger under these conditions. 3) It was established that the rate of delivery could be adjusted to allow enough level so that delivery rates were in the range of 12 ul/100g body weight of experimented animal. This was achieved.

It should be pointed out that the system needs close monitoring. Placement of the pipette tip against a tubule wall can cause partial blockage. In like manner, sediment in the pipette tip can cause similar problems. Each delivery should be timed and the volume monitored. There are some slight differences between pipettes, but whether this is a result of physical causes or of normal variability of measurement has not been established.

In October and November, Dr. Kowluru returned to my laboratory and we did two experiments/week. These consisted of infusions of test solutions into proximal tubules and monitoring the amount of test material which escaped absorption.

In November, Dr. Solomon was stricken with pneumonia and because of illness and vacations no further work was done until January. At this time the infusion studies were completed by S. Solomon. So that counting procedures would be constant, he brought all samples to Los Alamos, where Dr. Kowluru did the counting on the same equipment as used in October and early November.

Nothing further will be reported on the infusion studies since the results already have been prepared for publication. Data have been assembled, analyzed and the text written by Dr. Kowluru. The results can be summarized by stating that evidence was obtained indicating that glycosylated albumin is not absorbed while unmodified albumin underwent extensive absorption. This difference took place under both free flow and blocked tubule conditions.

This conclusion has been confirmed in tubule perfusion studies. In this experiment, a tubule is blocked with oil and vented proximal to the block to allow escape of filtrate. The delivery pipette is inserted into the tubule at the distal end of the block. Perfusion is started and recollected in a second oil filled pipette, distally placed. After a sample is collected it is counted for I_{125} (protein) and H_3 (inulin as a volume change marker). From changes in the relative concentrations of counts, the absorption of protein relative to water was calculated.

Because of the obvious difficulty in doing these studies it took Dr. Solomon several weeks until he was satisfied that he was getting valid data.

These data are summarized in the appendix. As can be seen, there is absorption of unmodified albumin but not of glycosylated albumin. An obvious question about these data is that the ratio of I_{125}/H_3 using unmodified albumin is slightly but significantly greater than one. Since the experimental animal cannot manufacture I_{125} , the most likely explanations are 1) some kind of systematic counting error or 2) some slight selective loss of inulin through the tubule wall under these conditions. Neither of these alternatives seem likely to Dr. Solomon and further work is needed to establish if this difference is truly biologically real.

Certain other observations were made which were of interest. The amount of fluid which could be collected in a given time period was less with the unmodified albumin solution than when using glycosylated. The difference in volume is ascribed to the osmotic drag produced by the glycosylated albumin.

In a few instances it was possible to puncture the perfused tubule at two sites. The amount of unmodified albumin reabsorbed was less when collected close to the perfusion pipette than when the collection was made more distally.

Because funds had been depleted, and no other monies were available to provide additional support, the last experiment was done one May 17.

Prepared May 29, by Sidney Solomon, Ph.D. Prof. Emeritus of Physiology

Sidney Solomon Ph.D.

PERCENT RECOVERY OF ALBUMINS FROM PERFUSED PROXIMAL TUBULES

<u>Glycated Albumin</u>			<u>Unmodified Albumin</u>		
n ¹	Mean $\pm$ S.E	P mean -100	n	Mean $\pm$ S.E	P mean -100
22	126.9 $\pm$ 8.154	< .005	18	76.0 $\pm$ 7.835	< .01

Probability that glycated and unmodified albumins are handled the same is <.001

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ANNUAL REPORT OF WORK CARRIED OUT UNDER CONTRACT
WITH LOS ALAMOS NATIONAL LABORATORY

Period covered by this report February 1, 1988 - May 31, 1989

During the first month, a technician was hired, the equipment and supplies were assembled for doing micropuncture experiments and the technician was trained for doing analyses at the micro-level. With contributions made by Drs. Kowluru and Bitensky, new methods were developed for sharpening pipettes for micropuncture so that it became possible to complete as many as 30 pipettes/hour.

Dr. Kowluru then spent time in my laboratory learning the techniques he needed to carry out micropuncture on his own anion. For about one month, he was in Albuquerque three days a week. During this time he became quite adept at doing these studies and obtained data on filtration at the single nephron (SNGFR) level which was comparable to that found in the literature (For a summary, see ref. 1).

In this laboratory, we then asked the question, "Is there any change in the distribution of SNGFR when rats become diabetic after streptozotocin injection (STZ)?" To carry out this study, the procedure used in this laboratory was used. Briefly, both whole kidney filtration rate (GFR) and SNGFR are measured and GFR is divided by SNGFR. This yields a calculated number of nephrons per kidney. Since the actual number of nephrons in this strain is approximately 31,000 a number which is greater than this number shows a relative deficit in filtration by surface nephrons, while a low number shows

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excess filtration by superficial nephrons. The data are summarized in Table I. It can be seen that untreated controls have low superficial SNGFR. STZ treated rats which develop frank glucosuria show the highest superficial SNGFR. STZ treated rats without glucosuria have the lowest SNGFR but have an intermediate relationship in regard to relative filtration by superficial nephrons.

At this time, Ms. Miyashiro, who had been hired as a technician had to resign because of excessive demands of her schoolwork. I held the job open for her as long as possible. When it became irrevocable that she could no longer work in this laboratory, I transferred Mr. Leo Martinez into her position. He was trained to prepare pipettes and carry out analytical procedures.

Dr. Kowluru again visited our laboratory to learn the tubule perfusion technique. This is a most demanding procedure. Although Dr. Kowluru made remarkable progress, more practical work is needed before reliable data can be obtained. To this end, I have lent the Los Alamos Laboratory micropuncture equipment so that Dr. Kowluru can set up his own micropuncture station.

In our laboratory, microperfusion studies are being continued with the aim of determining absorption of unmodified and glucosylated albumin. As yet, it would be premature to discuss these results.

In addition to these activities, I would like to mention certain additional tasks which were performed. I have spent considerable time reviewing, studying, and thinking about the diabetes literature. In addition, I have participated in a number of conferences and discussions about the problems of interest to us both at Los Alamos and in Albuquerque.

Prepared June 19, 1989, by Sidney Solomon

A handwritten signature in cursive script, reading "Sidney Solomon".

Reference: 1 Solomon, S., J. Physiol. 272: 573-589, 1977.

APPENDIX I

Superficial nephron filtration rates of
control and streptozatocin treated rats

Treatment	N	$\text{nl}/\text{min}^{-1}$	$\text{ml}/\text{min}^{-1}$	GFR	SNGFR/100 g. BW
		SNGFR	GFR	<u>SNGFR</u>	
None	6	5.798	.5576	96,177	21.09
None	4	3.818	.4145	115,631	17.78
None	1	8.86	1.56	176,072	30.55
STZ (No gluc in Urine)	4	4.10	.6415	154,573	14,643
STZ (No gluc in Urine)	7	3.952	-	-	15.200
STZ (No gluc in Urine)	5	4.059	.1133	27,900	13.53
STZ (No gluc in Urine)	8	4.777	.3654	76,492	16.08
STZ (U gluc 2000 mg/dl)	7	9.833	.08906	9,057	42.02
STZ (U gluc 100 mg/dl)	6	16.705	.3479	20,826	57.60

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