

## THE INFLUENCE OF NITROGEN RETENTION UPON THE REGENERATION OF PLASMA PROTEINS

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(Received for publication, November 27, 1939)

There is an unanimity of opinion on both the experimental and clinical sides that cases of hypoproteinemia due to "loss and lack" of protein respond promptly to proper dietary intake of protein (1). Cases of Bright's disease, however, after a certain stage of the disease has been reached, a stage characterized by nitrogen retention and proteinuria, do not respond to protein feeding in a predictable manner. Keutmann and Bassett (6) have tested upon cases of Bright's disease some of the dietary factors found by Whipple and his coworkers (5, 13) to be potent for the formation of new plasma proteins in "standard hypoproteinemic dogs." While high protein feeding in the patient with nephritis caused considerable storage of nitrogen in the body, presumably in the form of protein, the plasma proteins were not increased. Proteinuria in some cases increased without a corresponding fall in the plasma protein concentration. Is this failure of the nephritic to respond to protein feeding as do cases of nutritional edema related to nitrogen retention? Specifically, is something lost in the urine or held back in the plasma as a result of the renal injury which upsets the normal mechanism for plasma protein formation? These are the questions which the present investigations sought to answer.

### *Methods*

The general plan of the experiments is merely a continuation of previous studies (5, 8, 9, 10, 13). With the experimental animals, normal adult dogs maintained on a standard low protein diet, the plasma proteins, including the reserve stores, are depleted to a basal level of 4 per cent by repeated bleedings and return of the washed red blood cells suspended in saline (plasmapheresis). This results in the standard hypoproteinemic dog whose rate of plasma protein production, basal output, on the standard low protein diet is surprisingly uniform. After this hypoproteinemic state has been established, uranium nitrate is injected subcutaneously and the effect of the ensuing elevation in N.P.N. upon plasma protein production is followed. If the dog with uranium injury behaves as does the patient with Bright's disease not only would the basal output drop sharply, but various dietary (5, 8, 13) and amino acid supplements (9), which have been shown to be efficient for plasma protein production in standard hypoproteinemic dogs, would be without effect.

The standard basal diet used in these experiments consisted of: calf liver (raw wet weight) 32 parts, cane sugar 25 parts, corn starch 25 parts, butter 12 parts, cod liver oil 6 parts. Enough tomato juice was added to make a paste of which each gram contained 3 calories. The diet is fed in amounts to furnish 75 calories per kilo per day. 1 gm. of McCollum-Simmonds salt mixture (8) and 5 gm. of kaolin were thoroughly mixed with each day's diet.

The methods for depleting the plasma proteins, establishing the standard hypo-proteinemic state, measuring the basal output, testing the "efficiency" of various supplements that may be added to the basal diet, etc., have recently been published in detail (10). The only departure from the procedure described is the use of 10 per cent trichloroacetic acid instead of the phosphotungstic acid mixture in the separation of protein N and N.P.N. Duplicate micro Kjeldahl analyses of total N, N.P.N., and albumin plus N.P.N. (the filtrate from 22 per cent  $\text{Na}_2\text{SO}_4$  precipitation by Howe's method) served as the basis for calculation of plasma protein removed and blood level studies (12). For urinary protein the method of Folin and Dennis was used (3). In fractionating the N.P.N. the following methods were used: urea N, Lieboff and Kahn (7), creatine and creatinine N, Hawk and Bergheim (4). Undetermined N was calculated by difference.

The uranium nitrate used was obtained from Merck,  $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  lot S-7497, and was injected subcutaneously in the groin in 0.5 per cent aqueous solution. Sufficient water was used to insure quantitative transfer.

#### EXPERIMENTAL OBSERVATIONS

The data pertinent to the question which these studies sought to answer, namely, does nitrogen retention influence plasma protein production, are presented in Charts 1, 2, and 3, and in Table I.

In the first experiment (Chart 1) on a 2 year old, female mongrel, weighing 5.8 kilos, during the first week of fasting, the plasma proteins fell from 7.4 to 7.1 per cent; and during the next 4 weeks with removal of 3480 cc. of blood and 2040 cc. of plasma containing 91 gm. of plasma protein, the plasma protein level was reduced to 4 per cent. The reserve amounted to 31 gm. During the following week, the removal of 12 gm. of plasma protein was sufficient to maintain the basal level; and this was recorded as the basal output. The N.P.N. increased from 30 to 45 during the week of fasting, and declined to 25 at the end of the period of basal output. At this time 3.0 mg. of uranium nitrate per kilo was injected subcutaneously in the right groin. The N.P.N. rose progressively during the next 2 weeks to 386 before the animal died in uremia. Despite the removal of 21 gm. of plasma protein during this 2 week interval, which added to the 2.8 gm. of protein lost in the urine equals basal expectancy, the plasma protein level rose sharply to 6.5 per cent just before death. Some of this increase may have been due to hemoconcentration. It is safe to say, however, that there is no evidence of interference with plasma protein formation; if anything, the contrary is true.

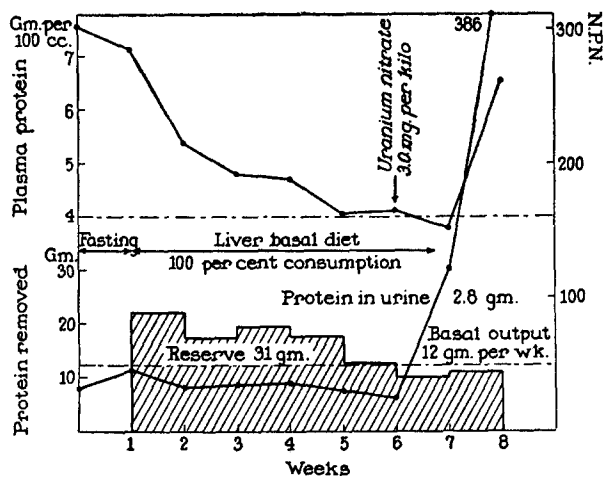


CHART 1. Dog 38-24. Female mongrel, 2 years, 5.8 kilos.

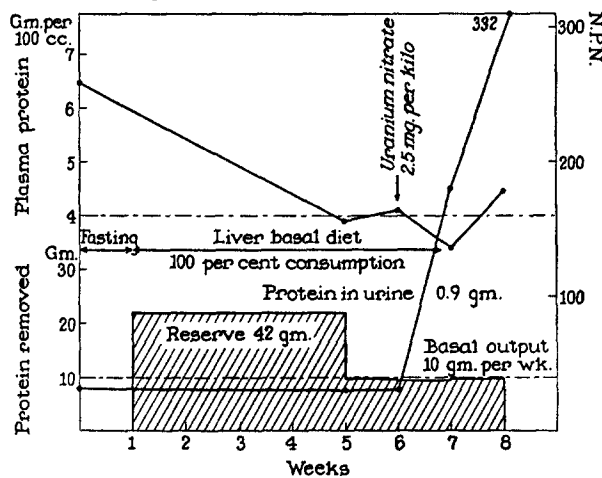


CHART 2. Dog 38-25. Female mongrel, 3 years, 4.8 kilos.

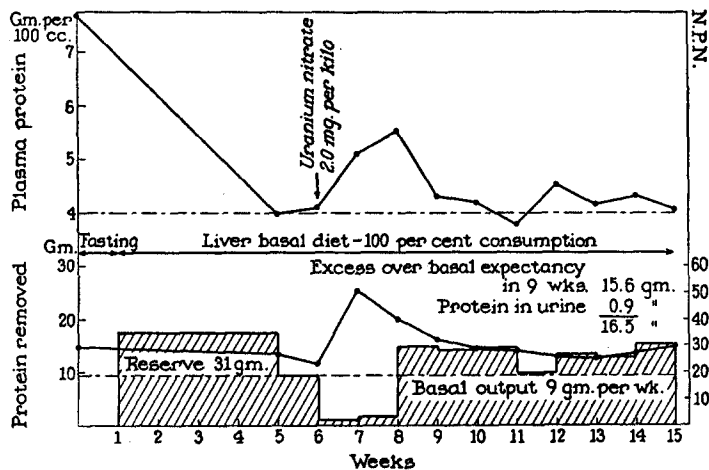


CHART 3. Dog 39-27. Female mongrel, 1½ years, 4.0 kilos.

The next experiment (Chart 2) is similar. After exhaustion of a reserve of 42 gm. (removal of 3650 cc. blood and 2045 cc. plasma containing 92 gm. of plasma protein in 23 exchanges) the basal output was found to be 10 gm. for this 3 year old female mongrel weighing 4.8 kilos. The N.P.N. remained constant at 28 mg. per 100 cc. until 2.5 mg. per kilo of uranium nitrate was injected subcutaneously at the end of the period of basal output. Thereafter the N.P.N. of the blood rose sharply to 332 mg. per 100 cc. before the animal died. During these 2 weeks the expected basal output of plasma protein was removed (if we include the 0.9 gm. of protein lost in the urine). Despite this removal the final concentration of plasma protein was above the basal level. There was no evidence of hemoconcentration in this dog. During the 2 weeks preceding death, roughly twice the total amount

TABLE I  
*Fractionation of Non-Protein Nitrogen before and after Administration of Uranium Nitrate to Standard Hypoproteinemic Dogs*

Dog No.	N.P.N.	Before	1 week after	2 weeks after
38-25	Total	32.3	145.0	332.0
	Urea N	18.7 (58)	70.3 (48)	126.5 (38)
	Creatine and creatinine N	1.7 (5)	6.6 (5)	13.8 (4)
	Undetermined N	11.9 (37)	68.1 (47)	191.7 (58)
39-27	Total	23.0	50.5	39.6
	Urea N	11.6 (51)	26.1 (52)	18.1 (46)
	Creatine and creatinine N	1.5 (6)	1.9 (4)	1.6 (4)
	Undetermined N	9.9 (43)	22.5 (44)	19.9 (50)

of circulating plasma protein was removed; this had to come from the basal diet, or from body protein, and did so at the expected rate of basal output. Thus again extreme elevation in N.P.N. did not affect the plasma protein forming mechanism.

The third and last experiment (Chart 3) is somewhat different. Only 2.0 mg. per kilo of uranium nitrate was given, as it was evident from the two previous experiments that the dog with depleted plasma proteins is more susceptible to uranium nitrate injury than is the normal dog which usually survives 4.0 mg. per kilo. The elevation in N.P.N. in this experiment is less marked—only about twice the normal level—but the experiment was continued over a period of 9 weeks. Plasmapheresis was discontinued during the 2 weeks after the injection and the plasma protein level rose to 5.5 per cent. During the next 7 weeks an excess over basal expectancy for the 9 weeks of 15.6 gm. of plasma protein had to be removed to keep the

plasma protein level near 4 per cent. The N.P.N. returned to normal after about 4 weeks. Again there is no evidence from this experiment that there was any interference with plasma protein production as a result of the renal injury with nitrogen retention.

Table I shows the fractionation of the N.P.N. in two of the dogs before and after the administration of uranium nitrate. It was thought that these fractions might give some indication as to the mechanism of any disturbance in the plasma protein forming mechanism. Despite the fact that no such disturbance developed, these fractions are of interest in showing that even marked shifts in the urea N and especially in the undetermined N fractions do not upset the plasma protein forming mechanism.

#### DISCUSSION

The experimental data indicate conclusively that even marked elevations of N.P.N. do not affect plasma protein formation in the standard hypo-proteinemic dogs. The significance of this negative finding for human cases of nephritis is not clear. One possible interpretation is that the diminished ability of the nephritic to form new plasma proteins is related to a more general disturbance of metabolism in which the elevation in N.P.N. is secondary. This focuses attention on what happens to the plasma protein forming mechanism when "nephrosis" becomes "nephritis." In this connection it must be remembered that the clinical and anatomical picture produced by uranium nitrate is that of nephrosis; but there is one significant difference from human cases of nephrosis, the elevated N.P.N. Whether this fact is sufficient to make the experimental animals comparable to patients with nephritis is open to question. About all that can be stated from the data at hand is that neither elevation of N.P.N. nor proteinuria *per se* upsets the plasma protein forming mechanism.

In harmony with these observations are those of Whipple and Robscheit-Robbins (14) who showed that neither elevation of N.P.N. nor proteinuria seriously interferes with the production of another blood protein, hemoglobin. Standard anemic dogs which develop "spontaneous nephritis" with proteinuria and nitrogen retention are still capable of producing hemoglobin at the rate of 70 per cent of normal expectancy, even in advanced stages of the disease.

#### SUMMARY

1. Because of the clinical observation that the capacity to form new plasma proteins is sometimes impaired in cases of nephritis (2), experiments

were performed to determine whether the impaired function in the nephritic is related to nitrogen retention.

2. These experiments consisted of producing renal injury by injecting uranium nitrate into standard hypoproteinemic dogs and comparing the rate of blood plasma protein formation under these conditions of nitrogen retention with that in the uninjured dog.

3. Despite elevations in blood N.P.N. to more than ten times normal, no interference with plasma protein formation was observed. These elevations in N.P.N. affected principally the urea and undetermined fractions.

4. Neither elevation in N.P.N. nor proteinuria *per se* appears to have any effect upon plasma protein production. Possibly the deficient production of plasma proteins in the nephritic is related to a more general disturbance in metabolism in which the elevation in N.P.N. is secondary.

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