4. Kawin, B., *Biol. Research Annual Rep.,* Hanford Atomic Products Operation, HW-47500, 1956, pp74.
5. Catsch, A., *Experientia,* 1957, v13, 312.
6. MacDonald, N. S., Spain, P. C., Ezmirlian, F., Rounds, D. E., *J. Nutrition,* 1955, v57, 555.
7. Wasserman, R. H., Schooley, J. C., Comar, C. L., *Midyear Report. Oak Ridge Inst. Nuclear Studies Publ. ORINS*-16, 1956, p24.
8. Comar, C. L., Whitney, I. B., Lengemann, F. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 232.
9. Comar, C. L., Wasserman, R. H., Nold, M. M., *ibid.,* 1956, v92, 859.
10. Wasserman, R. H., Comar, C. L., Papadopoulcu, D., *Science,* 1957, v126, 1180.
11. Palmer, R. F., Thompson, R. C., Kornberg, H. A., *ibid.,* 1958, v127, 1505.

Received September 25, 1959. P.S.E.B.M., 1960, v103.

## Erythropoietic Factor in Kidney Tissue of Anemic Dogs.* (25435)

JEAN-PIERRE NAETS† (Introduced by John H. Lawrence)
*Donner Lab., and Lawrence Radiation Lab., University of California, Berkeley*

In previous communications it was shown that bilateral nephrectomy abolished erythropoiesis in the dog(1) and that ureter ligation did not impair this function despite the same degree of uremia and malnutrition(2). In other studies an erythropoietic factor was demonstrated in plasma and urines of bled dogs(3) and disappearance of this factor was shown to occur after bilateral nephrectomy (4). These results agree with experiments by Jacobson *et al.*(5) on the rat and suggest that the kidney is the source of an erythropoietic stimulating factor. Several attempts to point out the erythropoietic factor in acidified boiled filtrates from kidney and other organs(6,7,8) have been unsuccessful. This study was an attempt to demonstrate erythropoietic stimulating activity in kidney homogenates.

*Materials and methods.* Fifteen mongrel dogs weighing between 13 and 25 kg were used. Three were acutely bled to a hematocrit of 14—9% in 6 hours at which time they were bled to death by femoral puncture. Twelve were made anemic (hematocrit 19—5%) by bleeding for 3 to 5 days. In this group 6 dogs were unilaterally nephrectomized 10 to 20 days before bleedings; the removed normal kidneys were used as normal controls. Blood volume was maintained by Dextran infusion as described elsewhere(3). Some dogs died from anemia and the kidneys were taken immediately after death; in other experiments the kidneys were removed under nembutal anesthesia before the animal's death. In 8 experiments the liver was also removed and in 5 the spleen. Removed organs were kept frozen until used. Kidney, liver and spleen tissue were ground with saline in a Waring Blendor for 3 to 5 minutes; saline volume added 150% of the weight of each organ; the tissue suspension was filtered through gauze and injected subcutaneously into rats without further treatment. Erythropoietic activity of organ suspensions was measured by $Fe^{59}$ red cell incorporation assay on starved rats(9), as described in previous paper(3). Recipient rats received 3 ml of organ suspension subcutaneously daily for 2 successive days before $Fe^{59}$ injection. Four to 10 rats were used for each determination. Controls consisted of uninjected rats and groups receiving normal kidney suspension, "anemic" liver suspension and "anemic" spleen suspension. After organ emulsion injections all rats developed abscesses at the site of injection and of the 253 rats used, 26 died from the toxicity of the material injected; no difference in toxicity was noticed for the extracts from different tissues. Hematocrits were measured in Wintrobe tubes by centrifugation at 3,000 rpm for 30 minutes. Hemoglobin was deter-

* This study is based on work performed under contract with U. S. Atomic Energy Comm.

† Charles Pecher post-doctoral Fellow at University of California on Fulbright Travel Grant. Present address: Brugmann University Hospital, University of Brussels.

TABLE I. Effect of Ground Tissue Injection on $Fe^{59}$ Uptake into Red Cells of Starved Rats.

| | Donor dogs | | % $Fe^{59}$ uptake into red cells of recipient rats | | | | | |
|---|---|---|---|---|---|---|---|---|
| No. | Hemato-crit, % | Hemoglo-bin, % | Uninj. control | Normal kidney inj. | "Anemic" kidney inj. | "Anemic" liver inj. | "Anemic" spleen inj. | Duration of bleeding |
| 34 | 5 | 2.2 | 5.6 ± 1.4 (6)† | 6.0 ± 2.8 (5) | 9.9 ± 4.7 (5) | | | 4 days |
| 38 | 14 | | 4.6 ± .8 (6) | 8.0 ± 4.2 (5) | 16.7 ± 7.9 (9) | | | 6 hr |
| | | | | 4.7 ± 3.3 (7) | | | | |
| 40 | 9 | | 4.6 ± .3 (10) | 8.3 ± 1.8 (8) | 12.2 ± 5.8 (8) | 8.3 ± 4.6 (8) | | 6 " |
| 41 | 16 | 4.8* | 5.1 ± 2.8 (9) | 7.1 ± 2.7 (7) | 20.3 ± 6.1 (8) | | 6.5 ± 2.1 (4) | 3 days |
| 45 | 11 | 3.6 | | | | | | |
| 42 | 19 | 5.8 | 3.8 ± .7 (9) | 14.6 ± 4.0 (8) | 11.4 ± 3.5 (7) | | | 3 " |
| 46 | 11 | 3.2 | *Idem* | *Idem* | 20.5 ± 11.6 (8) | 10.6 ± 4.1 (8) | 20.1 ± 8.9 (8) | 4 " |
| 44 | 11 | 5.2 | 3.3 ± .6 (10) | 7.6 ± 2.8 (6) | 10.9 ± 3.3 (8) | 11.4 ± .3 (8) | | 6 hr |
| 47 | 14 | 5.8 | 5.3 ± 1.9 (10) | 9.3 ± 2.1 (8) | 11.5 ± 7.0 (6) | 11.7 ± 5.8 (7) | 8.5 ± 3.9 (7) | 4 days |
| | | | | 13.6 ± 5.8 (8) | | | | |
| 48 | 9 | 3.2 | *Idem* | *Idem* | 14.2 ± 7.1 (6) | | | 5 " |
| 50 | 8 | 3.8 | 4.9 ± .6 (10) | 9.6 ± 4.8 (8) | 11.3 ± 7.1 (7) | | | 5 " |
| 57 | 8 | 3.4 | *Idem* | *Idem* | 19.8 ± 5.2 (7) | 10.3 ± 3.8 (7) | | 5 " |
| 55 | 7 | 2.0 | 4.8 ± .6 (7) | 11.4 ± 2.3 (6) | 13.5 ± 5.1 (8) | 8.6 ± 3.7 (6) | | 5 " |
| 61 | 8 | 3.8 | 4.9 ± .7 (6) | | 17.5 ± 3.3 (4) | | | 4 " |
| 64 | 8 | | *Idem* | | | 14.0 ± 3.9 (6) | 10.0 ± 5.4 (7) | 4 " |
| 65 | 8 | 2.8 | " | | 20.1 ± 3.9 (4) | 9.7 ± 5.8 (5) | 4.5 ± 1.6 (4) | 3 " |
| | Mean | | 4.7 ± .9 | 9.1 ± 4.5 | 15.0 ± 7.2 | 10.6 ± 4.4 | 9.9 ± 7.9 | |
| | t | | | 4.05 | | 2.92 | 2.4 | |
| | p | | | <0.001 | | <0.01 | 0.02 < p < 0.05 | |

* Livers from dogs No. 41 and 45 were combined, and so were the kidneys. Figures in parentheses indicate No. of rats in each assay.
† Stand. dev.

TABLE II. $Iron^{59}$ Uptake into Red Cells of Starved Rats Compared with Iron Plasma Level of the Same Blood Samples.

| | Normal kidney | | "Anemic" kidney | | "Anemic" liver | | "Anemic" spleen | | Control uninj. | |
|---|---|---|---|---|---|---|---|---|---|---|
| Donor dog No. | Fe plasma level (pool values), μg % | $Fe^{59}$ up-take, % | Fe plasma level (pool values), μg % | $Fe^{59}$ up-take, % | Fe plasma level (pool values), μg % | $Fe^{59}$ up-take, % | Fe plasma level (pool values), μg % | $Fe^{59}$ up-take, % | Fe plasma level (pool values), μg % | $Fe^{59}$ up-take, % |
| 47 | 142 | 9.3 (8) | 126 | 11.5 (6) | 112 | 11.7 (7) | 164 | 8.5 (7) | | |
| 48 | 93 | 13.6 (8) | 100 | 14.2 (6) | | | | | | |
| 55 | 62 | 11.4 (6) | 62 | 13.5 (8) | 39 | 8.6 (6) | | | | |
| 57 | 104 | 9.6 (8) | 95 | 19.8 (7) | 119 | 10.3 (7) | | | | |
| 50 | | | 93 | 11.3 (7) | | | | | | |
| 61 | | | 178 | 17.5 (4) | | | | | | |
| 64 | | | | | 152 | 9.7 (5) | 158 | 10.0 (7) | 276 | 4.9 (6) |
| 65 | | | 129 | 20.1 (4) | 95 | 14.0 (6) | 138 | 4.5 (4) | | |
| Avg | 100 | 11.0 | 115 | 15.9 | 103 | 10.9 | 153 | 7.7 | 280 ± 38* | 4.7† |

* Avg of 10 individual measurements; other iron plasma assays are made with pooled plasma. Figures in parentheses indicate No. of rats used in each assay.
† Mean of 83 control uninj. rats used.

mined by the method of Crosby (Cyanmethemoglobin method)(10). Plasma iron level determinations were made by technic of Peters *et al.*(11).

*Results* are summarized in Table I. Rats injected with "anemic" kidney tissue showed larger uptake of $Fe^{59}$ into red cells than those injected with normal kidney, "anemic" liver and "anemic" spleen. After "anemic" kidney injection the uptake was 15.0% (from 9.9 to 20.5%) while after normal kidney, "anemic" liver or "anemic" spleen injections it averaged 9.1% (4.7 to 14.6%), 10.6% (8.3 to 14.0%), and 9.9% (4.5 to 20.1%) respectively.

The significance of the difference in $Fe^{59}$ uptake, between groups of rats injected with "anemic" kidney emulsion and rats receiving other tissue emulsions, is indicated at the bottom of Table I. After only 6 hours bleeding (dogs No. 38, 40 and 44), "anemic" kidney suspension induced increased $Fe^{59}$ uptake. Twenty-six control rats were injected with normal kidney and compared with 25 rats receiving "anemic" kidney from dogs No. 38, 40 and 44 (Table I); iron uptake was 7.1% $\pm$ 3.1 after injection of normal kidney and 13.4% $\pm$ 6.6 after "acute anemic" kidney administration. The difference between these two values (anemia of 6 hours duration) is significant.

*Discussion.* It can be seen from the Table that $Fe^{59}$ uptake is similar after injection of normal kidney, "anemic" liver and "anemic" spleen but higher than iron uptake observed in uninjected starved rats. This increased iron uptake seems to be nonspecific and could be related to the caloric content of the administered material(9). Nevertheless the caloric content of 3 ml of organ suspension (which corresponds to 1.2 g wet tissue) is low, about 2 calories. It is unlikely that "anemic" kidney is more calorigenic than normal kidney or "anemic" liver.

The possibility exists that injected foreign proteins lower the plasma iron level in the recipient rats, thus increasing the $Fe^{59}$ uptake into red cells(12). In order to test this possibility, plasma iron level was measured in pooled rat plasma obtained by centrifugation of blood taken for $Fe^{59}$ uptake measurements. The results are presented in Table II. After tissue injections the plasma iron level was lower than in uninjected starved rats. This lower plasma iron level, together with the few calories injected could explain the higher $Fe^{59}$ uptake observed after injection of organ suspensions. On the other hand, Table II demonstrates that injected anemic kidney tissue did not decrease plasma iron level of the recipient rats more than normal kidney and "anemic" liver injections. This suggests that the higher iron uptake obtained after anemic kidney injection was related to an erythropoietic factor present in the tissue. This factor does not come from the anemic plasma contained in the kidney since even if very active the amount of contained plasma was too small to induce increased $Fe^{59}$ uptake. Injections of 2 to 6 cc of anemic dog plasma are required to induce increased iron uptake in starved rats(3,4); furthermore, "anemic" liver and "anemic" spleen contain about the same amount of active plasma as "anemic" kidney.

Although erythropoietic activity of the kidney of anemic dogs was not sufficiently greater than that of other tissues to demonstrate conclusively the importance of the kidney in erythropoietin production, it was sufficient to indicate that with adequate methods of isolating and concentrating the activity from crude homogenates a conclusive demonstration might be possible. Fractionation procedures are being investigated.

*Summary.* Homogenates of kidney, liver and spleen tissue from normal and anemic dogs were assayed for erythropoietic activity by their effect on $Fe^{59}$ red cell incorporation in starved rats. A slightly higher incorporation resulted from administration of an extract of anemic kidney than from extract of normal kidney or anemic liver or anemic spleen. This difference was sufficient to suggest the importance of developing methods for concentrating and purifying the erythropoietic activity of tissue homogenates to help clarify the role of kidney in production of erythropoietin. Methods for fractionation and concentration are being investigated.

1. Naets, J. P., *Nature,* 1958, v181, 1134.
2. ———, *ibid.,* 1958, v182, 1516.

3. ———, PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 387.

4. ———, *Nature*, 1959, v184, 371.

5. Jacobson, L. O., Goldwasser, E., Field, W., Plzak, L., *ibid.*, 1957, v179, 633.

6. Gordon, A. S., Piliero, S. J., Medici, P. T., Siegel, C. D., Tannenbaum, M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 598.

7. Piliero, S. J., Medici, P. T., Gordon, A. S., unpublished observations.

8. Rambach, W. A., Alt, H. L., Cooper, J. A. D., *Blood*, 1957, v12, 1101.

9. Fried, W., Plzak, L., Jacobson, L. O., Goldwasser, E., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 237.

10. Crosby, W. H., Munn, J. I., Furth, F. W., *U. S. Armed Forces Med. J.*, 1954, v5, 693.

11. Peters, T., Giovanniello, T. J., Apt, L., Ross, J. F., *J. Lab. Clin. Med.*, 1956, v48, 280.

12. Hodgson, G., Eskuche, I., Yudilevich, D., Hernandez, P., Tcha, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 826.

Received September 25, 1959. P.S.E.B.M., 1960, v103.

## Further Studies on Effects of Tranquilizing Drugs on Mammary Involution in the Rat.* (25436)

G. K. BENSON (Introduced by R. Gaunt)

*Nat. Inst. for Research in Dairying, Shinfield, Reading, Berkshire, England*

Oxytocin administration inhibits mammary involution in rats after removal of the litter at fourth day of lactation, and it was suggested that oxytocin elicits prolactin release under these conditions(1,2). Later, following reports of its lactogenic effects in several species (3-7), it was shown that reserpine resembles oxytocin in showing a striking effect in retarding mammary involution in the rat(8). It was of interest to study effects on mammary involution of other tranquilizing drugs, and the present paper describes experiments carried out with chlorpromazine, reported to cause galactorrhoea in women(9,10), and syrosingopine (Singoserp), an analogue of reserpine possessing hypotensive properties similar to those of reserpine, but with a considerably reduced central sedative action(11,25). To throw light on the mechanism of action of reserpine, the effects of hypophysectomy and of adrenalectomy on reserpine-induced parenchyma maintenance have been investigated. Further, in view of claims that the pharmacological action of reserpine may be due to altering the serotonin-binding capacity of brain cells(12,13), the effects of serotonin on mammary involution have been studied; and since reserpine has been reported to release histamine from blood platelets(14), the effect of this substance on mammary involution was also investigated.

*Materials and methods.* Hooded Norway rats were used, 2-3 months old, undergoing first lactation. Stock colony diet(15) was fed *ad lib.*; daily food and water consumption was recorded. After parturition all litters were reduced to 9 young on first day of lactation, and to 8 on second day. Litters were weighed daily to check lactational performance of the mother, and mothers were also weighed daily as in previous experiments. Litters were removed on 4th day of lactation when treatment began and when operations, if any, were carried out. Substances administered during treatments were a) Reserpine (Serpasil†), b) Syrosingopine (Singoserp‡), c) Chlorpromazine (Largactil, May & Baker Ltd.), d) 9 $\alpha$ fluorocortisol (FC) supplied as acetate by E. R. Squibb & Sons, N. J.; this was administered in suspension in 0.5 ml carboxy-methylcellulose (CMC) solution, e) Serotonin, supplied as creatinine sulphate by May & Baker Ltd., f) Histamine, supplied as acid phosphate by British Drug Houses Ltd. Full details of experimental groups, dosages,

* The author is indebted to Ciba Inc., N. J., through courtesy of Dr. R. Gaunt, for grant to cover cost of publication.

† Kindly supplied by Mr. C. W. S. Taylor, Ciba Labs., Horsham, Sussex.

‡ Kindly supplied by Dr. R. Gaunt, Ciba Inc., Summit, N. J.