ine, adenosine, hypoxanthine, and inosine, but not by guanine and guanosine. DAP potentiated cytotoxicity of 2-deoxy-D-glucose and hadacidin as was observed previously for certain other compounds which stimulated glucose utilization and lactic acid production.

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1. Mandel, H. G., *Pharmacol. Rev.*, 1959, v11, 743.

2. Burchenal, J. H., Bendich, A., Brown, G. B., Elion, G. B., Hitchings, G. H., Rhoads, C. P., Stock, C. C., *Cancer*, 1949, v2, 119.

3. Biesele, J. J., Berger, R. E., Hitchings, G. H., *Cancer Res.*, 1950, v10, 204.

4. Wheeler, G. P., Skipper, H. E., *J. Biol. Chem.*, 1953, v205, 749.

5. Remy, C. N., Smith, M., *ibid.*, 1957, v228, 325.

6. Neuman, R. E., Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 622.

7. Oyama, V. I., Eagle, H., *ibid.*, 1956, v91, 305.

8. Park, J. T., Johnson, M. J., *J. Biol. Chem.*, 1949, v181, 149.

9. Barker, S. B., Summerson, W. H., *ibid.*, 1941, v138, 535.

10. Neuman, R. E., Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 252.

11. Kaczka, E. A., Gitterman, C. O., Dulaney, E. L., Folkers, K., *Biochemistry*, 1962, v1, 340.

12. Neuman, R. E., Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 627.

13. ———, submitted for publication.

14. Shigeura, H. T., Gordon, C. H., *Cancer Res.*, in press.

15. Hakala, M. T., Taylor, E., *J. Biol. Chem.*, 1959, v234, 126.

16. Neuman, R. E., Tytell, A. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 763.

17. Shapira, J., Bornstein, I., Wells, W., Winzler, R. J., *Cancer Res.*, 1961, v21, 265.

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Relationship of Erythropoietin to Renal Juxtaglomerular Cells.* (27951)

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The arguments for the existence of a hematopoietic stimulating hormone, erythropoietin, have recently been reviewed(1). The evidence that erythropoietin is produced, at least in part, by the kidney is conclusive(2, 3,4,5,6,7,8,9). The exact cellular site of production within the kidney is uncertain. Reissman *et al.* postulated that the decreased erythropoietin levels in rats poisoned with bichloride of mercury point to a tubular site of production(5). Kuratowska *et al.* suggested renal vascular endothelium as the source(10). Osnes(7,11) has suggested the juxtaglomerular apparatus.

The latter hypothesis is of great interest in connection with the evidence, recently reviewed(12,13) that the juxtaglomerular cells located in the walls of the afferent arterioles, are stretch receptors and therefore sensitive to changes in arteriolar blood volume. It seems likely that this cell and its contiguous structure, the macula densa, are the major sources of renin(13). In the rat the renin content of the kidney varies directly with the degree of granularity of the juxtaglomerular cells(13).

In situations with a decrease in arterial blood volume, it would be extremely useful if this "volume receptor" secreted a hormone which stimulated erythrocyte production, and thereby increased the cellular element of the blood volume, in addition to its previously postulated effects on plasma volume and peripheral resistance. These effects operate either directly(14,15,16) or indirectly *via* aldosterone(17,18,19,20). Some evidence for such a relationship between erythropoietin and the juxtaglomerular cell has been reported by Hirashima and Takaku(21) who

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noted an association between increases in juxtaglomerular granularity and elevated plasma erythropoietin levels in rats made anemic by acute hemorrhage or injections of phenylhydrazine. Conversely, both juxtaglomerular granularity and erythropoietin levels were decreased below normal in rats made polycythemic by transfusion. Takaku *et al.* also found in rats that constriction of one renal artery is associated with increased levels of plasma erythropoietin(22); this procedure increases juxtaglomerular granularity in the kidney in which the artery is constricted(13). Finally, Fisher and Crook demonstrated that angiotensin stimulates erythropoiesis in hypophysectomized, adrenalectomized rats(23). The juxtaglomerular apparatus could well be related to this observation, since the renin liberated from it is necessary for production of angiotensin(24). To study this problem further, we elected to determine the erythropoietic activity of renal extracts from kidneys with varying degrees of juxtaglomerular granularity. Previously, Naets had shown that renal extracts from anemic dogs, with increased blood levels of erythropoietin, can stimulate erythropoiesis in assay animals(2). In the following experiments, we produced a high degree of juxtaglomerular granularity by placing rats on a diet low in sodium(13). The opposite condition, a low degree of juxtaglomerular granularity, was obtained either by using the contralateral unclipped kidneys of rats made hypertensive by clipping one renal artery or by using kidneys from rats given desoxycorticosterone trimethylacetate and 1% saline (13). Kidneys from rats on a high sodium intake would also have a moderate drop in justaglomerular granularity(13). If the juxtaglomerular cells did, in fact, produce erythropoietin, extracts from highly granulated kidneys might be expected to have more erythropoietic activity than those from kidneys with a low degree of granularity.

Methods. In *Experiment I,* to provide kidneys of varying degrees of juxtaglomerular granularity, 100 g male Wistar rats were divided into 5 groups. Group 1 was placed on a synthetic low sodium diet (0.005% sodium) for 10 weeks. Group 2 was placed on ordinary Purina laboratory chow for 10 weeks. Group 3 was placed for 10 weeks on a diet similar to that of Group 1 except that it contained 7.0% NaCl. Group 4 was given 2 subcutaneous injections of 50 mg of desoxycorticosterone (DOC) trimethylacetate with an interval of 4 weeks between injections. These rats ate ordinary laboratory chow and drank only 1% saline. Their kidneys were extracted 3 weeks after the second DOC injection. Group 5 was placed on normal chow for 10 weeks after having had a silver clip applied to one renal artery in order to narrow it. Arterial blood pressures, using the Friedman microphonic method with light ether anesthesia(25), were twice determined during the tenth week on Group 5. Only those rats with 2 systolic pressures of 150 mm Hg or more were used in the experiment. This level is quite hypertensive, since average blood pressure for normal rats of this age and strain is about 110 mm Hg (SD $\pm$ 8).

In the erythropoietin bioassay procedure, one or 2 rats of each group were killed. Both kidneys from each rat were used in Groups 1-4. Only the unclipped kidneys were used in Group 5. The kidneys were stripped of their capsules and homogenized in cold isotonic saline (1.5 cc/g of kidney) in a Potter-Elvehjem homogenizer. The mixture was then centrifuged at 1500 rpm for 30 minutes while being maintained at 15°C. The supernate was separated and immediately injected into the assay rats. For the erythropoietin assay 150-200 g male Wistar rats were used. Rats were starved a total of 72 hours, although water was given *ad libitum.* 2.5 cc of supernate from the kidney homogenization were injected subcutaneously after 24 and 48 hours of fasting. Control rats received injections of 2.5 cc of saline. After 54 hours of fasting 0.0001 mg of radioactive ferrous citrate (Fe^{59}) was injected into the saphenous vein. After 72 hours of fasting, the rats were bled by aortic puncture, and 4 cc samples were counted in a well type scintillation counter. Hematocrits were determined on these samples after counting. Red blood cell uptake of Fe^{59} was determined by the following equation(26).

$$\text{Uptake} = \frac{(\text{Counts/ml whole blood/min}) \left(\frac{\text{Pre-fasting wt}}{100} \times 2.21 \right)}{(\text{Hematocrit of sample})/(\text{Inj counts/min})}$$

In *Experiment II,* the same procedure as that of *Exp. I* was followed except for the fact that the assay rats were starved 96 hours instead of 72. Kidney injections were given at 24 and 48 hours after start of the starvation period. Fe^{59} was given at 78 hours, and rats were bled at 96 hours.

In *Experiment III,* 300 g male Wistar rats were placed on either low sodium (.005% Na) or high sodium (7.0% Na) diets for 6 weeks. The kidneys were then removed, and the rats were bled by aortic puncture using heparinized syringes (20 units of heparin/cc of blood). The plasma was separated by centrifuging at 15°C for 45 minutes at 2500 rpm, pooled, and stored at –26°C to be used one week later in *Exp. IV.* The kidney homogenates were prepared as previously described. The assay was carried out as described in *Exp. II,* except that the kidney injections were 3 cc each time instead of 2.5 cc. Each assay rat received kidney homogenates from 2 donor rats each day.

In *Experiment IV,* pooled plasma obtained in *Exp. III* was thawed and immediately assayed as described in *Exp. II.* Three cc injections of pooled plasma were given subcutaneously after both 24 and 48 hours of fasting.

Results. The results of *Exp. I* are given in Table I. Average uptake of Fe^{59} was considerably higher in Group 1 than in any of the other groups, while Groups 2-6 did not seem to differ from each other. For statistical purposes iron uptake values from Groups 2-5 were pooled and compared to Group 1. The difference was significant with a p value of 0.016.

The results of *Exp. II* are summarized in Table II. Again the kidneys from rats eating a diet low in sodium had more erythropoietic activity than any of the other groups, while Groups 2-5 did not significantly differ from one another. When Group 1 was compared to the pooled results of Groups 2-5, the difference was again significant ($p = .016$). Hematocrits of 12 of the rats on a low sodium diet whose kidneys were used in

TABLE I. (Exp I) Erythropoietic Activity of Extracts of Rat Kidneys with Varying Degrees of Juxtaglomerular Granularity.

Groups of rats providing kidneys for extraction	No. of assays	Avg uptake of iron in %	Stand dev of uptake	Avg hematocrit of assay rats at end of exp	Stand dev of hematocrit
1) Low sodium diet	10	44.1	± 16.2	43.7	± 3.3
2) Normal "	10	28.4	± 9.9	44.2	± 1.7
3) High sodium "	10	32.4	± 13.5	43.7	± 2.9
4) Rats made hypertensive by clipping one renal artery	10	32.4	± 6.0	43.5	± 2.3
5) DOC injected rats	9	27.1	± 18.0	45.6	± 2.2
6) Saline blank	10	29.5	± 20.8	49.6	± 6.0
7) Pooled results of 2, 3, 4, 5	39	30.9	± 9.4	44.2	± 2.8

TABLE II. (Exp II) Erythropoietic Activity of Extracts of Rat Kidneys with Varying Degrees of Juxtaglomerular Granularity.

Groups of rats providing kidneys for extraction	No. of assays	Avg uptake of iron in %	Stand dev of uptake	Avg hematocrit of assay rats at end of exp	Stand dev of hematocrit
1) Low sodium diet	5	39.4	± 19.9	44.2	± 4.0
2) Normal "	5	21.0	± 7.6	44.2	± 3.9
3) High sodium "	6	18.7	± 10.0	45.7	± 2.0
4) Rats made hypertensive by clipping one renal artery	5	14.8	± 4.7	44.0	± 4.6
5) DOC injected rats	6	22.6	± 16.9	44.6	± 2.1
6) Saline blank	10	12.5	± 4.0	43.0	± 2.9
7) Pooled results of 2, 3, 4, 5	22	19.4	± 11.5	44.6	± 3.0

Exp. I and *II* ranged from 41-49 with a mean of 46. This indicates that the increased erythropoietic activity of the renal extracts from these rats was not due to anemia.

In *Experiment III* average iron uptakes of the assay rats given renal extracts from rats fed either a low or a high sodium diet were 18.3% (S.D. ± 5.7) and 15.1% (S.D. ± 5.2) respectively. The difference was not significant ($p = 0.19$).

In *Experiment IV* average iron uptakes of the assay rats given plasma from rats fed low and high sodium diets were 18.9% (S.D. ± 5.2) and 19.8% (S.D. ± 6.7) respectively. This difference was not significant. Again, in the latter 2 experiments there was no anemia in either dietary group. Hematocrits of 12 low salt rats ranged from 43-53 with a mean of 47. Hematocrits of 12 high sodium rats ranged from 40-46 with a mean of 45.

The hematocrits of all the assay groups receiving kidney extract were virtually identical at the end of the experiments and well within the normal range. This eliminates the possibility that differential hemolytic effects of the various extracts were responsible for differences in iron uptake.

Discussion. These data indicate that extracts of kidneys with high degrees of juxtaglomerular granularity resulting from a low salt intake have increased erythropoietic activity. The extracts from the older 300 g rats on a low sodium diet did not have as great an increase in erythropoietic activity as did those from younger 100 g rats, possibly because the low salt diet is associated with higher degrees of juxtaglomerular granularity in younger rats than in older animals (27). The data also show that there is no statistically significant decrease in erythropoietic activity when extracts of kidneys from DOC injected rats and extracts of contralateral unclipped kidneys from rats with renal hypertension are compared to extracts of kidneys from rats on a normal diet. Yet the degree of juxtaglomerular granularity is very much less in the former than the latter (12). In *Exp. II* there actually was a considerable difference in erythropoietic activity between extracts of contralateral unclipped kidneys from rats with renal hypertension and extracts of kidneys from normal rats. Average uptake in the former was 14.8%, while that of the latter was 21.0% ($p = 0.16$). Perhaps if the number of rats used in the assay had been larger, the p value might have been smaller. Thus, while higher than normal degrees of juxtaglomerular granularity were associated with higher than normal erythropoietic activity, lower than normal degrees of granularity were not definitely associated with lower than normal erythropoietic activity. This would suggest that the juxtaglomerular granules themselves do not contain ordinary erythropoietin.

Although the results show some type of relationship between juxtaglomerular granularity and erythropoietin, the question as to the source of renal erythropoietin remains unanswered.

Summary. Erythropoietic activity of saline extracts of rat kidneys with varying degrees of juxtaglomerular granularity was studied. It was found that higher than normal degrees of juxtaglomerular granularity were associated with higher than normal erythropoietic activity. Lower than normal degrees of juxtaglomerular granularity were not definitely associated with lower than normal erythropoietic activity. The evidence here does not conclusively prove or disprove that the juxtaglomerular apparatus secretes erythropoietin.

DOC Trimethylacetate was kindly furnished by Dr. Robert Gaunt, CIBA Pharmaceutical Co.

1. Gordon, A. S., *Physiol. Rev.,* 1959, v39, 1.

2. Naets, J. P., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 129.

3. Goldwasser, E., Fried, W., Jacobson, L., *J. Lab. & Clin. Med.,* 1958, v52, 375.

4. Gallagher, N., McCarthy, J., Lange, R., *ibid.,* 1961, v57, 281.

5. Reissman, K. R., Nomuro, T., Gunn, R. W., Brosius, F., *Blood,* 1960, v16, 1411.

6. Kuratowska, Z., Lewartowski, B., Michalak, E., *ibid.,* 1961, v18, 527.

7. Osnes, S., *Brit. Med. J.,* 1959, No. 5153, 650.

8. Jacobson, L., Goldwasser, E., Fried, W., Plzak, L., *Nature* (Lond.), 1957, v179, 633.

9. Fisher, J., Birdwell, B., *Acta Haemat.* (Basel),

1961, v26, 224.

10. Kuratowska, Z., Lewartowski, B., Michalak, E., *Bull. Acad. Polish Sc.,* 1960, v8, 77.

11. Osnes, S., *Brit. Med. J.,* 1960, No. 5180, 1153.

12. Tobian, L., *Physiol. Rev.,* 1960, v40, 280.

13. ———, *Circulation,* 1962, v25, 189.

14. Helmer, O. M., *The Second Hahnemann Symposium on Hypertensive Disease,* Philadelphia, May, 1961.

15. Masson, G. M. C., *ibid.*

16. Tobian, L., Coffee, K., Ferreira, D., Meuli, J., *J. Clin. Invest.,* 1962, v41, 1404.

17. Genest, J., Biron, P., Boucher, R., Kiow, E., Nowaczyski, W., Chretien, M., *The Second Hahnemann Symposium on Hypertensive Disease,* Philadelphia, May, 1961.

18. Spink, W. W., Vick, J., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 521.

19. ———, *ibid.,* 1961, v107, 777.

20. Bein, H. J., Jaques, R., *Experientia,* 1959, v16, 24.

21. Hirashima, K., Takaku, F., *Blood,* 1962, v20, 1.

22. Takaku, F., Hirashima, K., Nakao, K., *J. Lab. & Clin. Med.,* 1962, v59, 815.

23. Fisher, J., Crook, J., *Blood,* 1962, v19, 557.

24. Smeby, R., Bumpus, M., *The Second Hahnemann Symposium on Hypertensive Disease,* Philadelphia, May, 1961.

25. Friedman, M., Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 670.

26. Reichlin, M., Harrington, W., *Blood,* 1960, v16, 1298.

27. Hartroft, P. M., *First Hahnemann Symposium on Hypertensive Disease,* Philadelphia, Dec. 1958.

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Factors Influencing Pathogenicity of *Mycoplasma arthritidis* (PPLO).* (27952)

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Mycoplasma, (pleuropneumonia-like organisms, PPLO) pathogenic in many animal species, were first reported to be a causal agent of rat polyarthritis in 1939(1). In a previous study(2), rats bearing a lymphosarcoma developed sporadic polyarthritis during or after regression of the tumor. When PPLO were recovered from tumor or joint lesions, and injected into PPLO-free rats, polyarthritis was produced in those rats with necrotic or recently regressed tumors, but not in normal rats or in those with intact tumors. Additional studies suggested that both host and agent factors were significant in the pathogenesis of the PPLO polyarthritis(3). As one phase in the investigation of the pathogenesis of mycoplasmal arthritis, the present study concerns the modification of virulence of Mycoplasma *in vitro* as well as *in vivo*.

Materials and methods. PPLO, recovered from a joint lesion of a rat with a regressed Murphy-Sturm lymphosarcoma, were maintained by infecting the transplantable lymphosarcoma in young male Holtzman rats, 120 to 170 g in weight, as previously described (2). PPLO from tumor tissue were isolated on blood agar plates. Small segments of agar with large numbers of PPLO colonies were inoculated into Difco PPLO broth. Broth subcultures were made at weekly intervals using a 20% inoculum from the previous subculture and incubating for 5 to 7 days at 37°C. Aliquots were injected intravenously into rats, 0.5 ml/100 g body weight. The criterion for mycoplasmal pathogenicity was the production of gross joint lesions.

Tumor extract was prepared by aseptically removing growing lymphosarcoma, mincing in an equal volume of balanced salt solution (Earle's), and grinding in a Waring blendor. Tissue fragments, cells and extraneous bacteria were removed by Seitz filtration. The tumor extract was added as a supplement in 10% concentration to Difco PPLO broth

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