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CHEMICALLY-INDUCED RENAL HYPERTROPHY IN THE RAT

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Abstract—The renohypertrophic effects of folic acid have been compared with those of a number of other compounds of related or unrelated structure, and with other stresses on the kidney such as those caused by ureteral ligation or the administration of uranium salts. It is suggested that the renohypertrophic effects of folic acid and other compounds are the result of partial tubular blockage caused by precipitation of the substances in the kidney tubules.

THE ABILITY of the pteridine, xanthopterin to cause a temporary increase in kidney size and an associated burst of mitotic activity in the renal tubules was first reported by Haddow[1] who subsequently found a number of other compounds, including the substituted pyrimidine compound 2:4:5-triamino-6-styryl-pyrimidine, were also capable of causing renal enlargement.[2–4] More recently it has been shown that folic acid, which contains a substituted pteridine nucleus similar to that of xanthopterin, also initiates increases in the rates of growth and of RNA and DNA synthesis in the rat kidney.[5–8]

The aim of the present work is to examine some possible mechanisms by which folic acid and other compounds exert their effects on the kidney. In the experiments described the effectiveness of folic acid in inducing renal hypertrophy and increased nucleic acid synthesis has been compared with that of a number of other compounds including related pteridine compounds such as tetrahydrofolic acid and methotrexate. In addition the response of the kidney to stresses such as ligation of the ureter or the administration of uranium salts has been compared to that of folic acid. The results of these experiments and of measurements of the retention of folic acid in the kidneys are discussed and a mechanism for the renohypertrophic action of folic acid and other compounds is proposed.

MATERIALS AND METHODS

Animals. Male rats of the highly-inbred August strain, or occasionally male F_1 hybrid rats bred from August and Marshall parents, were used. The animals which were 6–8 weeks old at the start of each experiment were maintained throughout on M.R.C. Diet No. 41B, free access to both food and water being allowed at all times.

Administration of drugs. Folic acid (British Drug Houses Ltd) (Poole, Dorset,

England) and Methotrexate (a gift from Lederle Laboratories, London) were injected i.v. as solutions in 0·3 M sodium bicarbonate, 5:6:7:8-Tetrahydrofolic acid (Sigma Chemical Co. Ltd., London) was dissolved in 0·3 M sodium bicarbonate solution, containing 0·05 M ascorbic acid to minimize oxidation of this unstable derivative of folic acid, and injected i.v. immediately after preparation of the solution.

Triamteren (2:4:7-triamino-6-phenyl-pteridine) was purchased from the Aldrich Chemical Co., Milwaukee, U.S.A; xanthopterin and the substituted pyrimidines were obtained from Koch-Light Laboratories Ltd., Colnbrook, England. The 2:4:5-triamino-6-styryl-pyrimidine was a gift from Professor W. C. J. Ross of the Chester-Beatty Research Institute. These compounds were administered by i.p. injection as approximately neutral aqueous solutions or suspensions, or as suspensions in arachis oil.

The amounts of the pteridine or pyrimidine administered were 560 μmole/kg body wt. unless otherwise indicated.

Uranyl nitrate (Hopkins & Williams Ltd., Chadwell Heath, England) was injected i.v. in aqueous solution at a dose level of 4 μmole/kg body wt.

Ureteral ligation. A dorso-lateral incision was made under ether anaesthesia and the left kidney was exposed. The left ureter was tied off with a single ligature placed about 1 cm below the hilum. The kidney was then replaced and the incision closed with sutures and clips.

Determination of nucleic acids. RNA and DNA fractions were prepared from kidney homogenates by a modified Schmidt-Thannhauser procedure and determined by u.v. spectrophotometry as described previously.[6]

Measurement of rate of DNA synthesis. The rate of DNA synthesis in the kidney at various times after the injection of the drugs was measured by determination of the spe. act. of DNA extracted from the kidney 1 hr after the i.p. injection of 0·5 μ Ci/g body wt. of tritiated thymidine (Radiochemical Centre, Amersham, England); sp. act. approximately 10 Ci/m-mole.

Assay of radioactivity. Radioactivity measurements were carried out with a liquid scintillation counter (L.S.C.30 Tracerlab S.A., Malines, Belgium) using an emulsion counting technique.[9] The scintillator liquid was prepared by mixing 7 vol. of NE 214 liquid scintillator (Nuclear Enterprises (G.B.) Ltd., Edinburgh, Scotland) with 3 vol. of Triton X 100 (a gift from Lennig Chemicals Ltd., London). 1 cc of the DNA extract in 1 N perchloric acid mixed with 10 cc of scintillator mixture was counted with an efficiency of about 12 per cent.

Retention of folate in the kidney. The gross retention of folic acid in the kidney was measured at various times after administration by a simple spectrophotometric procedure.

Single kidneys were homogenized in 1 M Tris-HCl buffer at pH 8·8 using an M.S.E. Homogenizer (Measuring & Scientific Equipment Ltd., London, England). The homogenate was diluted to 50 cc with the buffer and centrifuged at 105,000 *g* for 30 min. The absorption spectrum of the supernatant, or a suitable dilution, was then measured over the range 350 to 450 mμ in a Cary 15 recording spectrophotometer. (Applied Physics Corporation, Monrovia, California, U.S.A.). The typical absorption spectrum showed a peak or, at high folate concentrations, a point of inflexion at 412 mμ and a second peak due to folate at 365 mμ. Corrections for non-folate absorption in the 365 mμ band and folate absorption in the 412 mμ band were made.

The accuracy of this procedure is about 20 per cent and the limit of detection about 2 μg/ml of homogenate.

A similar procedure was used for the measurement of methotrexate retention in the kidney.

RESULTS

The wet and dry weight, RNA and DNA contents of the kidneys of rats measured 4 days after administration of various pteridine or substituted pyrimidine compounds are listed in Table 1 and the effects of uranyl nitrate and unilateral ligation of the ureter on these parameters are shown in Tables 2 and 3. The time interval of 4 days was chosen as this represented the time of maximum increase in kidney size after administration of folate or xanthopterin but this might not in fact be the optimum time for measurement of the effects of some other substances.

TABLE 1. WEIGHT AND NUCLEIC ACID CONTENT OF RAT KIDNEYS 4 DAYS AFTER ADMINISTRATION OF PTERIDINE OR PYRIMIDINE COMPOUNDS (560 μmole/kg BODY WT.)

Compound	Route of injection	No. of rats	Wet wt. mg*	Dry wt. mg*	RNA μg*	DNA μg*
None	—	33	4·35 ± 0·45	0·96 ± 0·10	26·2 ± 1·5	20·6 ± 1·9
Xanthopterin 2-Amino-4:6:-dihydroxypteridine	I.P.	6	6·88 ± 0·80	1·33 ± 0·10	47·6 ± 5·0	32·6 ± 2·2
Folic acid	I.V.	20	7·56 ± 1·20	1·28 ± 0·17	51·8 ± 7·9	34·3 ± 4·5
Tetrahydrofolic acid	I.V.	5	4·40 ± 0·19	0·89 ± 0·02	26·9 ± 0·7	20·7 ± 0·8
Triamteren 2:4:7-triamino-6-phenyl-pteridine	I.P.	6	4·35 ± 0·25	0·99 ± 0·03	27·9 ± 1·1	21·8 ± 2·6
2:4:5-Triamino-6-styryl-pyrimidine	I.P.	4	8·40 ± 0·38	1·31 ± 0·06	48·8 ± 1·2	38·6 ± 0·3
2:4:5-Triamino-6-hydroxy-pyrimidine	I.P.	5	4·18 ± 0·43	0·87 ± 0·09	—	—
2:4:6-Triamino-pyrimidine	I.P.	5	4·26 ± 0·44	0·94 ± 0·10	—	—
4:5:6-Triamino-pyrimidine	I.P.	5	4·07 ± 0·40	0·90 ± 0·10	—	—
2:4:5:6-Tetramino-pyrimidine	I.P.	3	4·55 ± 0·14	0·91 ± 0·01	27·8 ± 2·0	21·7 ± 2·6

* Data expressed as mg or μg/kidney/g body wt.

The effects on the rate of DNA synthesis in the kidney measured at 18, 24 and 48 hr after administration of four pteridine compounds are shown in Table 4.

The amounts, expressed as a percentage of the administered dose, of folate or methotrexate retained per kidney at various times after i.v. injection are illustrated in Fig. 1. The dose response relationships for folic acid and xanthopterin are shown in Fig. 2.

TABLE 2. WEIGHT AND NUCLEIC ACID CONTENT OF THE RAT KIDNEY 4 DAYS AFTER INTRAVENOUS INJECTION OF URANYL NITRATE (4 μmole/kg BODY WT.)

	No. of rats	mg/g body wt. Wet wt.	mg/g body wt. Dry wt.	μg/g body wt. RNA	μg/g body wt. DNA
No Treatment	8	4·40 ± 0·16	0·96 ± 0·04	25·2 ± 1·0	23·5 ± 1·00
Uranyl nitrate	8	6·27 ± 0·44	1·08 ± 0·05	30·6 ± 2·9	28·7 ± 1·8

TABLE 3. EFFECT OF LIGATION OF THE LEFT URETER ON THE WEIGHT AND NUCLEIC ACID CONTENT OF THE *LEFT* KIDNEY

Time after ligation	No. rats	mg/g body wt. Wet wt.	μg/body wt. RNA	μg/body wt. DNA
No ligation	3	3·21 ± 0·30	16·5 ± 1·2	13·1 ± 0·7
1 Day	4	3·89 ± 0·27	20·9 ± 1·1	13·7 ± 1·0
2 Days	4	4·11 ± 0·36	24·8 ± 1·5	15·4 ± 1·1
3 Days	5	4·21 ± 0·38	25·7 ± 2·3	17·0 ± 0·7

TABLE 4. EFFECT OF FOLIC ACID AND OTHER COMPOUNDS ON DNA SYNTHESIS IN RAT KIDNEY (560 μmole/kg BODY WT.)

(100 μCi ^{3}H-Thymidine administered 1 hr before sacrifice)

Compound	Sp. act., cpm/mg DNA 18 hr	24 hr	48 hr
None—control		3170 ± 940 (20)	
Folic acid	5490 ± 2400 (5)*	64300 ± 20470 (7)*	39800 ± 15300 (8)*
Tetrahydrofolic acid	—	5250 ± 1470 (5)*	3630 ± 1400 (5)
Xanthopterin	—	40620 ± 15400 (5)*	41280 ± 4740 (3)*
Methotrexate	3360 ± 1120 (4)	6750 ± 1770 (4)*	3400 ± 690 (8)

Figures in parenthesis indicate number of animals.

* Significantly greater than control, $0{\cdot}0005 < P < 0{\cdot}0025$.

DISCUSSION

Examination of the results listed in Table 1 shows that only three of the compounds listed, folic acid, xanthopterin and 2:4:5-triamino-6-styryl-pyrimidine, caused an increase in the wet and dry weight and RNA and DNA contents of the kidney when these parameters were measured 4 days after injection of the compound. Two other compounds, tetrahydrofolate and methotrexate, produced small but significant increases ($0{\cdot}0005 < P < 0{\cdot}0025$) in the rate of DNA synthesis at 24 hr (Table 4), but by 48 hr the rates of synthesis were indistinguishable from control values.

A comparison of the structural formulae of the compounds tested here, and of other compounds which were reported to be active by Haddow and his colleagues[1, 2] has failed to reveal any simple correlation between a specific chemical structure and renohypertrophic activity. Folic acid and xanthopterin, which have identical pteridine moieties, produce responses of the same magnitude when administered in equimolar

amounts (Fig. 2) whereas methotrexate, tetrahydrofolate and triamteren do not induce renal hypertrophy although their pteridine rings are only slightly different from those of folic acid and xanthopterin. Similarly 2:4:5-triamino-6-styryl-pyrimidine produces marked renal hypertrophy but 2:4:5-triamino-6-hydroxy-pyrimidine does not.

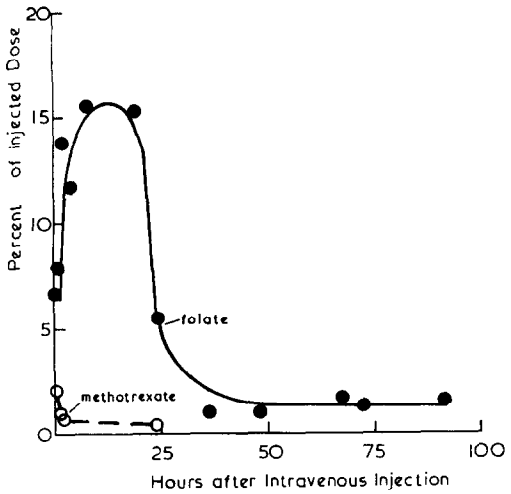


FIG. 1. The retention of folate and methotrexate in single rat kidneys at various times after i.v. injection of 560 μmole/kg body wt.

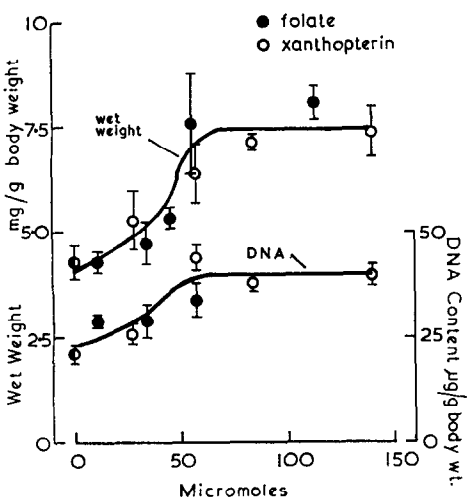


FIG. 2. The changes in the wet weight and DNA content of rat kidneys measured 4 days after varying doses of folate or xanthopterine.

The fact that xanthopterin is as active as folic acid in producing renal hypertrophy while tetrahydrofolate is inactive suggests that the observed effects do not result from a stimulation of the usual folate-catalysed biochemical reactions; since if this were so tetrahydrofolate should be active but xanthopterin should be inactive.

It would seem therefore that the common renohypertrophic action of the various active compounds does not result from a specific and direct effect on some biochemical system but probably arises from a non-specific effect on the kidney.

One property which is shared by the active compounds is a relatively low solubility at neutral or acid pH. Thus when a large amount of folate or other substance is filtered off by the kidney the pH fall along the tubule can cause the substance to precipitate resulting in partial or complete blockage.

Gross visual examination of the kidneys after administration of any of the active compounds shows the presence of considerable amounts of the solid drug. Microscopic examination of frozen sections shows that many of the tubules are filled with solid material (Fig. 3). Following treatment with any of the inactive compounds the kidneys appear normal on gross visual examination and no evidence of large amounts of solid material in the tubules is seen on microscopic examination. The presence of crystals of xanthopterin in the renal tubules was reported by Haddow.[2] However this author did not observe crystallization of 2:4:5-triamino-6-styryl-pyrimidine in the tubules. This failure to demonstrate 2:4:5-triamino-6-styryl-pyrimidine in the tubules could well have resulted from the material being leached out of the tissue during fixation in histological processing.

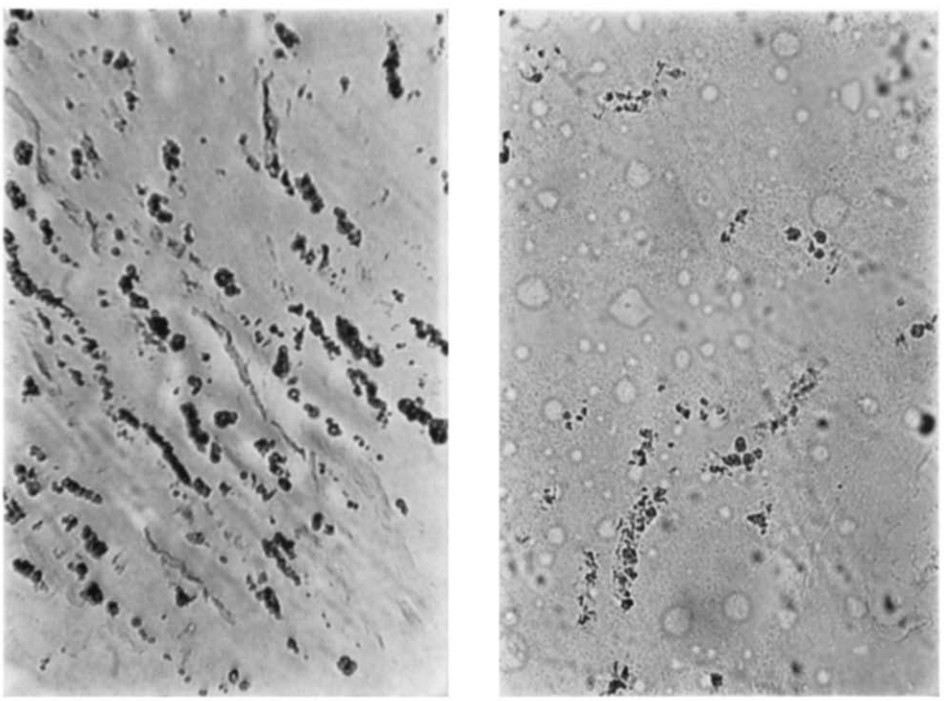
Tubular precipitation has also been suggested to explain the specific effects of uranium on the kidney tubules[10] and as can be seen from Table 2, the administration of uranyl nitrate causes a similar pattern of change in wet and dry weight and in RNA and DNA content to that produced by folate.

Such a blockage or partial blockage of the tubules would have the effect of markedly increasing the "functional load" on the remaining intact tubules. Thus the changes observed in the kidney after administration of folate or other active substances may represent a response to increased functional load. This is supported by studies on the effect of ligation of the left ureter on the weight and nucleic acid content of the left kidney. It can be seen from the data in Table 3 that ligation of the left ureter produces similar changes in the left kidney to those seen after folate administration. The right kidney of the ligated animals showed a similar pattern of change but the increases were smaller in magnitude.

Increased functional load in the remaining kidney has also been suggested as the main reason for the 'compensatory hypertrophy' which occurs after unilateral nephrectomy.[10] However it has been shown previously that the rate of response of the kidney to folate administration differs greatly from that seen after unilateral nephrectomy. One possible reason for some of these differences is that in the case of folate administration at the dose level used in these studies there is a sudden massive increase in the functional load of the kidney, while after unilateral nephrectomy the work load of the kidney only doubles. This sudden increase in 'functional load' following folate is indicated by the data on folate retention in the kidney which are shown in Fig. 1.

As much as 8 per cent, or 2 mg, of folate is present in each kidney as early as 30 min after folate injection, rising to values as great as 20 per cent, or 5 mgm/kidney at about 8 hr.

The failure of the folic acid analogue methotrexate to cause more than a transient increase in the rate of DNA synthesis measured 24 hr after injection is most probably due to the fact that methotrexate does not accumulate in the kidney to the same extent as folic acid. Measurement of the retention of methotrexate in the kidneys showed



(a) (b)

FIG. 3. (a) Tubular deposits of folic acid in unstained frozen sections of rat kidney prepared 24 hr after folate injection (× 400).

(b) Tubular deposits of 2:4:5-triamino-6-styryl pyrimidine in an unstained frozen section of rat kidney, 24 hr after injection. (× 400).

that the highest concentration occurred 30 min after injection and was only about 2 per cent of the administered dose, Fig. 1. By 24 hr less than 0·4 per cent of the injected methotrexate remained in the kidney, compared with about 6 per cent of the folate.

In the case of tetrahydrofolate the failure to cause renal hypertrophy is not easy to explain. Neal and Williams[12] have shown that the urinary excretion of tritiated folate and reduced folates by rats was essentially similar when doses of up to 2·5 μmole were injected i.v., however in our experiments the doses administered were about 20 times greater and it is possible that differences in the urinary excretion rates of folate and tetrahydrofolate may become evident at these high levels. The transient increases in the rates of DNA synthesis observed following tetrahydrofolate and methotrexate may have been caused by the presence, as an impurity, of small amounts of folic acid.

Bergel[13] has reported that triamteren did show a renohypertrophic effect. However in none of our experiments have we been able to demonstrate any effect of this compound on renal growth, even at higher dose levels than that reported in Table 1. In contrast to the active compounds we have examined triamteren is extremely insoluble at alkaline pH but shows increasing solubility as the pH falls to strongly acid values. Thus it seems unlikely that after i.p. injections large amounts of this compound will rapidly reach the kidney; further, triamteren would be less likely to precipitate in the tubules than any of the active compounds tested.

Johnson and Roman[11] have suggested that following unilateral nephrectomy the primary and predominant response is cellular hypertrophy. In the chemically induced renal growth discussed here some degree of cellular hypertrophy does occur, as evidenced by an increase in the RNA:DNA ratio,[6] but it cannot be described as the predominant response; indeed in the animals given uranyl nitrate no evidence of cellular hypertrophy was seen.

The hypothesis that the renohypertrophic action of folate and other 'active' compounds results from increased functional load due to tubular blockage does not throw any light on the mechanism of initiation of the increased rates of RNA, DNA and protein synthesis[13] which are found. With all the agents discussed one of the initial changes is a marked retention of water in the kidney. If this increased water retention is reflected by some increase in the intracellular water content, this could cause a decrease in the effective concentration of certain electrolytes or other substances which may act as a trigger for protein or RNA synthesis.

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