Inhibition by Food Restriction of the Renal Damage Due to Corticoid-Overdosage

The syndrome of nephrosclerosis with hypertensive cardiovascular disease, produced by mineralocorticoid-overdosage in the rat, is frequently used to test drugs (sodium-exchange resins, antihistaminics, chlorpromazine, rauwolfia), suspected of having a beneficial effect upon comparable disease-manifestations in man (for lit. see[1]). It must be kept in mind, however, that treatment with some of these drugs seriously interferes with the appetite of experimental animals. We have often noticed that in rats which refuse their food (e.g., because of some incidental disease), it is particularly difficult to produce renal and cardiovascular lesions with desoxycorticosterone; hence it seemed of interest to establish the effect of partial food restriction upon the evolution of this experimental syndrome.

Treatment with glucocorticoids tends to antagonize many of the pathologic lesions normally elicited by overdosage with mineralocorticoids[2], but in this respect the kidney represents a notable exception[1]. In rats in which nephrosclerosis is produced by desoxycorticosterone, the renal damage is further aggravated by cortisone or cortisol, even if the animals lose body-weight as a result of glucocorticoid-treatment[1].

In view of these facts, we decided to undertake experiments on rats overdosed either with desoxycorticosterone alone or with desoxycorticosterone plus cortisol, and to determine the effect of food restriction upon the resulting syndromes.

Experimental Materials and Techniques. Fifty female Sprague-Dawley rats, having an initial body-weight of 94–104 g, were sensitized to the production of nephrosclerosis by removal of their right kidney and subsequent administration of 1% NaCl as a drinking fluid. At the same time, the animals were subdivided into five groups, each consisting of ten rats with an average body-weight of 99 g.

Beginning on the day of the operation, all the animals, except the controls (Group I), were given daily subcutaneous injections of 1 mg of desoxycorticosterone acetate (DOCA) in the form of microcrystals, suspended in 0·2 ml of water containing a trace of "Tween 80". Groups II and IV received no other hormone-treatment, while Groups III and V were given simultaneous injections of 1 mg of cortisol acetate (COLA) in the same form. Groups I, II and III had unrestricted access to food ("Purina Fox Chow"), while in Groups IV and V food-intake was so restricted that the animals lost about $^1/_5$ to $^1/_4$ of their initial body-weight in the course of the experiment. The NaCl-solution was available to all animals without restriction throughout the experiment, and their voluntary fluid-intake was measured daily.

After 20 days of treatment all the animals were killed, their organs fixed in Susa-mixture, weighed and histologically examined.

Results. It will be seen from the Table that in the controls (Group I) and in the animals receiving DOCA alone (Group II) there was a considerable gain in body-weight during the 20 days of observation. In the latter group the kidney was much enlarged and there was also marked nephrosclerosis with an increase in fluid-intake and some myocarditis. Simultaneous treatment with COLA (Group III) resulted in a moderate loss of body-weight and a slight diminution of the renal hypertrophy, as judged by the absolute weight of the kidney, but the relative renal weight was not diminished and the nephrosclerosis was actually aggravated as a result of the additional glucocorticoid-treatment. The fluid-intake and the myocarditis were not markedly altered in Group III, as compared with Group II.

On the other hand, the animals in Group IV, which lost 20% of their body-weight as a result of the partial starvation, showed no trace of renal or cardiac lesions, no marked renal hypertrophy and no increase in fluid-intake.

Although the animals in Group V lost even more body-weight than those in Group IV, they did exhibit not only a percentual but even an absolute increase in renal weight and cardiac weight. At the same time, there was marked nephrosclerosis and some myocarditis, as well as a moderate increase in fluid-intake.

Discussion. From these experiments it is obvious that, in rats receiving an unrestricted food-intake, the renal damage produced by DOCA is further aggravated by COLA. Partial starvation completely inhibited the nephrosclerosis and myocarditis caused by DOCA-overdosage under our experimental conditions. On the other hand, in rats simultaneously overdosed with DOCA and COLA, restriction of food-intake exerted a much less pronounced inhibitory effect upon the resulting renal damage and virtually none upon the myocarditis.

[1] H. SELYE, *Stress. The Physiology and Pathology of Exposure to Systemic Stress* (Acta Inc. Med. Publ., Montreal, 1950); *First Annual Report on Stress* (Acta Inc. Med. Publ., Montreal, 1951). – H. SELYE and A. HORAVA, *Second* (1952) and *Third* (1953) *Annual Reports on Stress* (Acta Inc. Med. Publ., Montreal). – H. SELYE and G. HEUSER, *Fourth Annual Report on Stress* (Acta Inc. Med. Publ., Montreal, 1954); *Fifth Annual Report on Stress* (M D Publ. Inc., New York, 1955).

[2] H. SELYE, Exper. *11*, 35 (1955).

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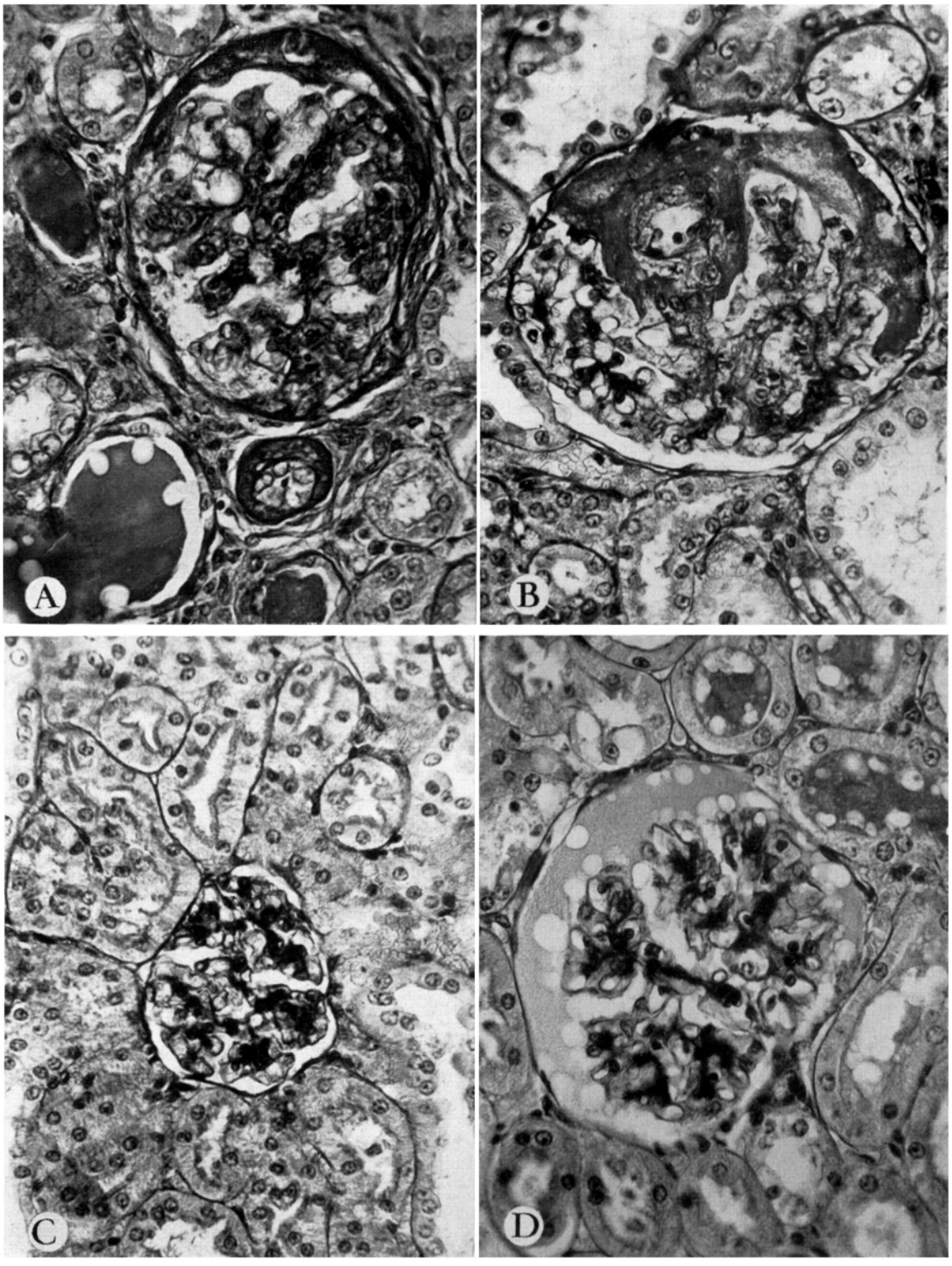
Group	DOCA (mg)	COLA (mg)	Food-Intake	Final Body-weight (g)	Weight Gain (%)	Kidney			Heart			Fluid-Intake (ml)
						Weight (mg)	(%)*	Nephro-Sclero-sis	Weight (mg)	(%)*	Myo-carditis	
I	0	0	Unrestricted	166	+66	1186 ± 32·0	717	0	636 ± 15·9	384	0	54 ± 4·3
II	1	0	Unrestricted	131	+32	1706 ± 60·5	1300	+ +	622 ± 5·8	474	+	112 ± 4·5
III	1	1	Unrestricted	90	− 9	1310 ± 72·0	1475	+ + +	472 ± 14·8	537	+	130 ± 8·4
IV	1	0	Restricted	79	−20	699 ± 22·7	878	0	360 ± 7·5	454	0	42 ± 3·3
V	1	1	Restricted	72	−27	955 ± 37·5	1376	+ +	392 ± 7·3	543	+	87 ± 5·2

* Organ-weight in mg/100 g body-weight.



Effect of food restriction upon renal damage due to corticoid-overdosage. *A* Kidney of rat receiving DOCA on an unrestricted diet (Group II). The glomerulus is greatly enlarged and partly hyalinized. The tubules are dilated and several of them contain hyalin-casts. *B* Kidney of rat receiving both DOCA and COLA on an unrestricted diet (Group III). Changes essentially similar to those in previous picture. *C* Treatment same as in "*A*" but the animal is kept on a restricted diet (Group IV). Renal structure essentially normal. *D* Treatment same as in "*B*" but the rat is kept on a restricted diet (Group V). Hyalinization is diminished, but there is still considerable accumulation of proteinaceous material in Bowman's capsule and there are hyalin-casts in the tubules.

We may conclude that—quite apart from their specific actions—drugs and other experimental conditions, which damage animals and thereby diminish their voluntary food-intake, may also inhibit the production of nephrosclerosis by corticoids through the partial starvation thus induced. As judged by the experiments

reported here, such a nonspecific antinephrosclerotic effect is less likely to occur, however, in animals receiving conjoint treatment with DOCA and COLA[3].

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Zusammenfassung

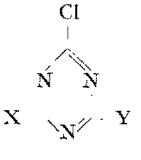
Bei Ratten, die durch einseitige Nierenexstirpation und 1% NaCl als Trinkflüssigkeit sensibilisiert wurden, war die Nephrosklerose, die normalerweise durch das Desoxycorticosteronacetat (DOCA) hervorgerufen wird, durch gleichzeitige Zufuhr von Cortisolacetat (COLA) noch verstärkt. Einschränkung der Nahrungszufuhr kann die Produktion von Nephrosklerose durch DOCA vollkommen verhindern, Unterernährung hat jedoch viel weniger Einfluss auf das Entstehen derartiger Nierenveränderungen nach gleichzeitiger Behandlung mit DOCA und COLA.

[3] This work was supported by a Grant from the Gustavus and Louise Pfeiffer Research Foundation.

Über Pflanzenwachstumsregulatoren*

Über weitere phytotoxische Triazine

In einer ersten Mitteilung[1] haben wir über die phytotoxischen Eigenschaften des 2-Chlor-4,6-bis-(diäthylamino)-s-triazins (G 25804) berichtet. Bei der Weiterbearbeitung dieser Körperklasse fanden wir nun einige Verbindungen[2], die eine wesentlich stärkere Aktivität aufweisen:



	X	Y	F	umkristallisiert aus:
G 27692	$-NHC_2H_5$	$-NHC_2H_5$	224 225°	Dimethylformamid[3]
G 27901	$-N(C_2H_5)_2$	$-NHC_2H_5$	101–103°	Äthanol[4]
G 28279	$-NH_2$	$-NHC_2H_5$	177–178°	Dioxan[5]
G 28509	$-NHCH_3$	$-NHC_2H_5$	236–237°	Dimethylformamid[6]
G 28510	$-NHCH_3$	$-NHC_3H_7$	203–205°	Äthylcellosolve

Die 2-Chlor-4,6-diamino-s-triazine lassen sich aus Cyanurchlorid durch Umsatz mit den entsprechenden aliphatischen Aminen leicht in guter Ausbeute erhalten. Sie sind als Chemikalien mit Ausnahme von G 28510 bereits beschrieben. Lediglich G 28279 hat eine Wasserlöslichkeit, die bei 20°C 1 ‰ übersteigt. Abgesehen von G 27901 sind sie in den üblichen organischen Lösungsmitteln bei Zimmertemperatur schwer löslich. Das Chloratom kann durch mässig konzentrierte Mineralsäure bei Wasserbadtemperatur hydrolysiert werden.

Fungizide und insektizide Eigenschaften wurden bei den genannten Verbindungen nicht oder in höchst geringem Masse angetroffen. Die Toxizitäten[7] gegenüber Warmblütern sind als gering zu bezeichnen, wie aus der folgenden Tabelle hervorgeht:

	DL 50 Maus per os		DL 50 Ratte per os	
G 27692	> 5	g/kg	> 5	g/kg
G 27901	1,75	,,	3,75	,,
G 28279	0,48	,,	0,48	,,
G 28509	2,5	,,	> 5	,,
G 28510	1	,,	2,2	,,

Zur ersten biologischen Beurteilung wurden dieselben Testmethoden benützt, wie wir sie in unserer ersten Mitteilung[1] beschrieben haben.

Es zeigte sich, dass auch diese Substanzen selbst *keinen Wuchsstoffcharakter* im klassischen Sinne aufweisen und den natürlichen Wuchsstoffhaushalt nicht stören. Waagrecht gelegte Bohnen, deren Epikotylunterseiten mit den Substanzen behandelt worden waren, entwickelten völlig normale negativ geotropische Reaktionen.

Dass auch die *Samenkeimung* praktisch nicht beeinflusst wird, geht aus Tabelle I hervor:

Tabelle I. Indexziffer: 10 = normale Keimung, 0 = keine Keimung, 9–1 = Zwischenstufen, über 10 = erhöhte Keimung.

Produkt	Testpflanzen		
	Hafer (*Avena sativa* L.)	Senf (*Sinapis alba* L.)	Gurken (*Cucumis sativus* L.)
G 27692	10	9	11
G 27901	10	8	12
G 28279	10	10	10
G 28509	10	10	11
G 28510	10	10	10
G 25804	10	10	12
2,4-D	10	1	5
CMU*	10	10	11
unbehandelt	10	10	10

* = 4-Chlorphenyl-dimethyl-harnstoff (Du Pont).

Die *Wirkung auf das Wachstum* der Keimpflanzen ist aus Tabelle II ersichtlich.

Die Beobachtungen liessen erkennen, dass die fünf Verbindungen eine ähnliche Wirkungsweise wie G 25804 aufweisen. Gramineen werden zuerst chlorotisch und sterben hernach ab. An Keimpflanzen von Senf werden als erste Vergiftungssymptome Dürrerscheinungen an den Rändern der Kotyledonen sichtbar; schliesslich vertrocknet die Pflanze, ohne dass eine Farbveränderung sichtbar wird. Das Absterben tritt in der Regel innerhalb drei Wochen ein. Im Vergleich zu CMU wirken die genannten Triazine etwas langsamer.

* 2. Mitteilung.

[1] A. GAST, E. KNÜSLI und H. GYSIN, Exper. *11*, 107 (1955).

[2] Verwendung zum Patent angemeldet.

[3] A. W. HOFMANN, Ber. deutsch. chem. Ges. *18*, 2755 (1885). – W. M. PEARLMAN und C. K. BANKS, Amer. Soc. *70*, 3728 (1948). – J. T. THURSTON *et al.*, Amer. Soc. *73*, 2981 (1951).

[4] W. M. PEARLMAN und C. K. BANKS, Amer. Soc. *70*, 3728 (1948).

[5] O. DIELS, Ber. deutsch. chem. Ges. *32*, 700 (1899). – W. M. PEARLMAN und C. K. BANKS, Amer. Soc. *70*, 3728 (1948).

[6] O. DIELS, Ber. deutsch. chem. Ges. *32*, 700 (1899).

[7] Wir verdanken die Bestimmungen Herrn Prof. Dr. DOMENJOZ.