Teneur en acide citrique (μg/g de tissu frais) des organes de la souris après injection de fluoroacétate (FA) et irradiation éventuelle à la 5e h (X).

	Souris témoins	5 h après FA	14 h après FA	14 h après FA, 9 h après X	9 h après X seuls
Reins	23	1333	1155	552	0
Myocarde	12	617	864	882	0
Rate	19	585	631	354	54(?)
Encéphale	20	111	88	62	12
Foie souris ♂	94	157	150	70	50
souris ♀	66	84	81	77	60

Souris C 57 noires de 18 à 22 g, nourries *ad libitum*. L'acide citrique est dosé par la méthode de TAYLOR[2]. Les valeurs de ce tableau sont des moyennes obtenues sur au moins 6 animaux. Les variations du taux d'acide citrique dans le foie des souris ♀ ne sont pas statistiquement significatives. L'effet antagoniste des rayons X (775 r, Stabilivolt Siemens 200 kV) sur l'accumulation d'acide citrique, produite dans le myocarde par injection de fluoroacétate aux animaux, n'apparaît qu'après la 14e h. La dose de fluoroacétate sodique utilisée (5 mg/kg) tue environ 30% des animaux dans les 3 jours.

et STOCKEN[3] ont constaté en effet que le foie des rats nourris accumule plus de citrate, après injection de fluoroacétate, que celui des rats maintenus à jeun 24 h avant l'injection du toxique. BUSCH *et al.*[4] ont montré d'autre part que des coupes de foie incubées en présence de fluoroacétate accumulent du citrate si elles disposent de substrats oxydables appropriés et si la pression partielle d'oxygène dans les coupes est suffisante.

L'influence des hormones sexuelles semble également évidente *a*) puisque les valeurs de citrate accumulé sont plus élevées dans le foie des rattes que dans le foie des rats[3], *b*) puisque la castration des rats mâles augmente leur capacité d'accumuler du citrate dans leur foie[5], tandis que la même opération sur les rattes produit plutôt l'inverse[6], *c*) puisque enfin l'accumulation de citrate dans le foie des rattes intoxiquées ne se manifeste qu'à l'âge adulte[6].

L'irradiation totale des rats diminue leur capacité d'accumuler du citrate dans leurs tissus sous l'influence d'une injection ultérieure de fluoroacétate[7]; cependant le foie des rats mâles accumule dans ce cas beaucoup plus de citrate[7]. Certains des effets immédiats et plus tardifs des irradiations pourraient être dûs à une action des rayons X sur les hormones androgènes[7].

Au cours de recherches entreprises en vue d'interpréter la radio-protection qu'assure à la souris l'injection préalable de doses sub-léthales de fluoroacétate[8], nous avons constaté que le foie des souris se comporte à l'opposé du foie des rats. Le foie des souris mâles accumule du citrate après administration de fluoroacétate; le foie des souris femelles ne le fait pas. Nous n'avons pas recherché l'effet du fluoroacétate à divers âges. Il est peu probable cependant que l'absence d'accumulation de citrate dans le foie des souris femelles soit dû au trop jeune âge des animaux; ces souris pesaient 18 à 22 g et devaient avoir plus de 12, sinon plus de 14 semaines.

L'irradiation des souris mâles 5 h après l'injection du toxique fait tomber leur taux de citrate hépatique à la moitié du taux du citrate hépatique de souris témoins intoxiquées mais non irradiées. Le foie des souris mâles répond par conséquent aux irradiations comme les autres tissus de cet animal[9]. Le foie du rat mâle par contre, accumule du citrate après empoisonnement au fluoroacétate si l'animal a été préalablement irradié[7]. La différence de comportement de ces deux espèces animales ne peut être dûe à l'âge de nos souris mâles: à 18–22 g, les souris mâles ont plus de 10 semaines et sont adultes. Remarquons que nous avons irradié les souris quelques heures après administration du poison alors que dans les expériences faites chez le rat[7], l'irradiation précédait l'administration de fluoroacétate.

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Summary

In contrast to rats, male mice injected with sodium fluoroacetate accumulate citrate in their livers, whereas females do not. Whole-body irradiation reduces the level of accumulated citrate in the liver as well as in other tissues of the mouse.

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The Oral Route for the Bio-Assay of Aldosterone-like Materials in the Rat: Relative Potency of Aldosterone and Chloro-Cortisol

Although numerous methods have been described (for reviews see [1]) for the bio-assay of aldosterone-like materials, no method for quantitative assay has been hitherto reported in which the steroids were administered by mouth. It seemed of interest to investigate the feasibility[2] of this route because (a) substances like liquorice and glycyrrhetinic acid have been reported[3] as having aldosterone-like activity in humans; and (b) the discovery[4] of new steroids capable of blocking the effects of aldosterone and DCA on electrolyte excretion makes it desirable to assess the administration by oral route in order to widen their possible therapeutic usefulness. It seems reasonable to postulate that any attempt to assay in the rat the oral effectiveness of an aldosterone antagonist on Na^+ and K^+, must be based on a satisfactory dose-

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Comparison of aldosterone-like effect between bio-assays by oral and subcutaneous route

Steroid	Oral				Subcutaneous			
Dose in μg	N[1]	k[2]	Response mean ± S. E.[3]	P<[4]	N	k	Response mean ± S. E.	P<
Aldosterone								
0·015	—	—	—	—	14	2	86·91 ± 7·47	0·2
0·05	—	—	—	—	14	2	59·86 ± 5·00	0·001
0·06	13	2	88·99 ± 7·40	0·3	—	—	—	—
0·15	—	—	—	—	14	2	33·55 ± 3·53	0·001
0·20	14	2	83·03 ± 7·67	0·05	—	—	—	—
0·50	—	—	—	—	14	2	22·27 ± 2·72	0·001
0·60	14	2	48·62 ± 5·08	0·001	—	—	—	—
2·00	14	2	29·11 ± 3·51	0·001	—	—	—	—
0·68	—	—	*50*	—	—	—	—	—
0·089	—	—	—	—	—	—	*50*	—
Regression line[5]	$y + 42{\cdot}27\,x = 42{\cdot}99$				$y + 43{\cdot}63\,x = 4{\cdot}29$			
S. D. L.[6]	8·90				6·28			
$S.E._b$[7]	6·99				4·93			
$L = \lvert b \rvert / S.D.L.$[8]	4·75				6·95			
r[9]	−0·97				−0·98			
Cl–F								
0·05	7	1	90·91 ± 19·05	0·7	7	1	79·47 ± 15·17	0·2
0·10	14	2	80·77 ± 14·37	0·2	7	1	68·26 ± 7·93	0·01
0·25	13	2	72·00 ± 9·34	0·01	6	1	37·89 ± 4·90	0·001
0·50	20	3	53·75 ± 3·59	0·001	5	1	35·58 ± 5·55	0·001
1·00	14	2	26·47 ± 0·92	0·001	—	—	—	—
5·00	6	1	13·25 ± 1·39	0·001	—	—	—	—
10·00	6	1	9·27 ± 1·70	0·001	—	—	—	—
0·59	—	—	50	—	—	—	—	—
0·20	—	—	—	—	—	—	50	—
Regression line	$y + 38{\cdot}40\,x = 41{\cdot}21$				$y + 48{\cdot}59\,x = 16{\cdot}38$			
S. D. L.	8·05				6·77			
$S.E._b$	3·88				8·90			
$L = \lvert b \rvert / S.D.L.$	4·77				7·19			
r	−0·97				−0·97			

[1] Total number of rats in dose assayed group. An equal number of rats was used as control for each group.
[2] Number of bio-assays.
[3] Standard error calculated as given by WORTHING and GEFFNER[10].
[4] Significance of difference between assay and control groups.
[5] x = log X; X = dose in μg.
[6] Standard deviation about the line.
[7] Standard error of slope.
[8] Estimate of index of precision.
[9] Coefficient of correlation.
[10] A. G. WORTHING and J. GEFFNER, *Treatment of Experimental Data* (Wiley, New York 1943), p. 207.

response relationship previously obtained by using aldosterone-like corticoids *per os* in the same type of bio-assay.

It was shown earlier[5] that aldosterone and 9α-chlorocortisol acetate (Cl–F) are very potent substances in promoting a fall of the urinary Na^+/K^+ ratio, the latter taken as an index of electrolyte-regulating activity in the rat. For this reason these corticoids were chosen for the present investigation. The method of bio-assay by subcutaneous route in the young adrenalectomized male white rat has been previously[6] described. For the oral route the same method was used, the only difference being that each steroid was administered by an intragastric tube in a volume of 0·15 ml (instead of 0·1 ml) of 20% ethanol/water as solvent. The activity of aldosterone and Cl–F was indicated by a fall in the Na^+/K^+ ratio in the urine of the rats, this ratio being expressed as a percentage of the mean for a control group. Thus a more intense effect is represented by a greater reduction of this percentage. Aldosterone was administered as D, L-aldosterone 21-mono-acetate. The *biological* activity of this chemical form is 4 tenths that of the non-acetylated D-aldosterone[7]. The doses of aldosterone listed in the Table express quantities of D-aldosterone, the amounts of the racemic aldosterone acetate actually given to each rat being therefore 2·5 times greater.

The results together with several calculated statistical parameters are shown in the Table. Values obtained by the

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[7] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Helv. chim. Acta *37*, 1163 (1954); Notice in J. clin. Endocrinol. *17*, 699 (1957).

subcutaneous route are also given for comparison. It was found that there is a linear relationship between the fall of the Na^+/K^+ ratio and the logarithm of the dose of each corticoid administered by mouth in the following ranges: aldosterone 0·06–2·00 μg; Cl–F 0·05–10·00 μg. As expected the response obtained by the oral route for each comparable dose level was less than that obtained when the same steroid was given subcutaneously. From the regression lines tabulated in the Table, the dose of each steroid which produces a 50% effect on the Na^+/K^+ ratio has been calculated, this being for the oral route 0·68 μg of aldosterone and 0·59 μg of Cl–F, and for the subcutaneous route 0·089 μg and 0·20 μg respectively.

Aldosterone, when administered by subcutaneous route, is at least twice as potent as Cl–F, as judged by comparison of the calculated dose of each steroid which produces a 50% response. In this context it is of great interest that aldosterone is *less* effective than Cl–F when given by mouth (Table). These observations reinforce the hypothesis[8] that the increased activity of some halogenated steroids as compared with 'natural' hormones may result from a slower rate of metabolization by the liver.

The conclusions to be drawn from this experiment are: (a) the oral route, though not as sensitive as the subcutaneous, can be used for the bio-assay of aldosterone-like materials in the rat; and by inference, for the assessment of aldosterone antagonists; (b) aldosterone is less effective by mouth than Cl–F; (c) the relative potency on electrolyte-regulating activity of two or more different steroids tested by the subcutaneous route should not be used as an indication of their relative potency by the oral route.

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Résumé

On démontre que chez le rat, l'administration orale de l'aldostérone et substances similaires pour essai biologique est pratiquable et sûre, bien que moins sensible que l'administration parentérale. Par inférence, il est suggéré que cette méthode est utilisable pour l'évaluation des antagonistes de l'aldostérone.

A l'inverse de leur efficacité relative par voie parentérale, l'aldostérone est par voie orale moins actif chez le rat que l'acétate de 9α-chlorocortisol pour produire l'abaissement du rapport Na^+/K^+.

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The Effect of 17α-Ethyl-19-Nortestosterone on the Estrus Cycle

17α-Ethyl-19-nortestosterone (Nilevar®)[1] is reported to show a potent anabolic[2], progestational[3], and deciduomagenic[4] activity in rats, an anti-estrogenic effect in spayed estrogen-treated rats[5], to inhibit the pituitary gland in parabiotic rats[6], and to damage the reproductive process as evidenced by the inhibition of the ovulation in rabbits[4] and by the antifertility[4] effect in rats. In the course of our investigations on the possibly protective effects of this new synthetic hormone in adult female, ethionine-treated intact rats, we have observed that the estrus cycle was completely inhibited during the time of the intramuscular administration of 0·5 mg/kg body weight, a dose being highly anabolic, but not yet androgenic[7]. Since the animals had to be killed after 13, or 22 days respectively, it cannot be said whether or when the normal cycle would have been restored after the medication has been stopped. Testosterone propionate in corresponding non-adrogenic doses[8], i.e. 0·04 mg/kg body weight, did not inhibit the estrus cycle: Only 2 out of 16 animals had a prolonged anestrus for the first 12, or 13 days respectively. All control animals treated with the vehicle (sesame oil) alone, showed no change of their normal cycles. The estrus cycle-interrupting effect of 17α-ethyl-19-nortestosterone can be explained by the known inhibition of the pituitary gland[6] and/or by the antimetabolite effect on the estrogens[5] present in the intact animal. Further detailed studies are in progress.

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Zusammenfassung

Im Verlaufe unserer Untersuchungen mit 17α-Äthyl-19-nortestosteron (Nilevar) beobachteten wir, dass bei erwachsenen weiblichen, mit diesem neuen Hormon behandelten Ratten kein Östrus mehr auftrat, während Testosteronpropionat in entsprechenden, nicht androgenen Dosen den östrischen Zyklus nicht signifikant beeinflusste.

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