

a single, close-packed unimolecular layer. While the detergent effect shows a dependence on detergent concentration, there exists a broad range of detergent concentrations over which the effect on the inhibition is essentially constant. For reasons indicated, the effect of

detergent has been provisionally interpreted as an effect on the interaction of EW inhibitor and vaccine.

The mechanism of the detergent effect is being investigated and will be the subject of a subsequent report.

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Assay for Antistiffness Activity with Guinea Pigs Depleted on Solid Rations.

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Oleson *et al.*¹ have described a solid basal ration with which they depleted guinea pigs for the purpose of conducting assays for anti-stiffness activity. Their results, however, seem to indicate shortcomings in the assay method. Thus, 13% of their negative controls showed marked improvement and an additional 17% showed slight improvement in stiffness. Furthermore, their homologous series of ergostanyl esters showed wide, inexplicable differences in activity.

Petering *et al.*² have recently described an apparently satisfactory assay method in which they used a solid, semipurified diet and with which they claim to have confirmed the anti-stiffness activity of ergostanyl acetate.

We have confirmed the production of wrist stiffness in guinea pigs with solid rations. Both the Lederle basal ration described by Oleson *et al.*¹ and the Rockland stock diet for guinea pigs[†] were successfully used to produce wrist stiffness. On the basis of our results, however, it is questionable whether the condition produced in guinea pigs on these rations is identical to the Wulzen stiffness syndrome which develops in animals fed the skim milk

diets.³⁻⁵ In our hands, in fact, these commercial, pelleted rations proved unsatisfactory for the assay of anti-stiffness activity.

That the condition which develops in guinea pigs on the Rockland and Lederle rations is not identical to that which has been produced by the Oregon group on skim milk diets is indicated by the fact that in our animals the easily hydrolyzable phosphorus of the liver did not decrease as drastically as in the experiments reported by van Wagtendonk⁵ and van Wagtendonk and Wulzen.⁶ In fact, we found no correlation between wrist stiffness and the easily hydrolyzable phosphorus of the liver in animals fed these rations.

We have nevertheless attempted to determine the antistiffness activity of a number of materials in assays involving several hundred animals fed these diets. Among the substances assayed were the previously reported active compounds listed in Table I.

Animals having a 3+ stiffness¹ were used for the therapeutic assays which, except for the diet, were conducted by the method of van Wagtendonk and Wulzen.^{4,6} Supplements were dissolved in cottonseed oil and adminis-

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¹ Oleson, J. J., Van Donk, E. E., Bernstein, S., Dorfman, L., and Subbarow, Y., *J. Biol. Chem.*, 1947, **171**, 1.

² Petering, H. G., Stubberfield, L., and Delor, R. A., *Arch. Biochem.*, 1948, **18**, 487.

[†] Arcady Farms Milling Company, Chicago, Ill.

³ Wulzen, R., and Bahrs, A. M., *Am. J. Physiol.*, 1941, **133**, P500.

⁴ van Wagtendonk, W. J., and Wulzen, R., *Arch. Biochem.*, 1943, **1**, 373.

⁵ van Wagtendonk, W. J., *J. Biol. Chem.*, 1944, **155**, 337.

⁶ van Wagtendonk, W. J., and Wulzen, R., *J. Biol. Chem.*, 1946, **164**, 597.

TABLE I.
Therapeutic Tests—Lederle Basal Ration.

Supplement	Dose 5x/week μg	No. of animals	Stiffness after 7 weeks			
			Improved	Slight or questionable improvement	No change	Worse
Ergostanyl Acetate*	5	19	1	2	2	15
	50	8	3	2	1	2
Ergostanyl Acetate†	5	18	2	1	2	13
	50	5	0	2	1	2
Natural anti-stiffness Factor‡	50	9	4	1	1	3
None	—	9	1	1	2	5
"	—	8	3	2	1	2

* Prepared by Dr. O. N. Breivik, Standard Brands, Incorporated.

† Supplied by Lederle Laboratories.

‡ Supplied by Dr. W. J. van Wagendonk.

TABLE II.
Ergostanyl Acetate*—8-Week Prophylactic Test—Rockland Diet.

Dose 7x/week, μg	No. of animals	Increase in stiffness		
		Marked	Slight	None
5000	10	4	1	5
500	10	6	3	1
5	6	3	2	1
0	15	6	2	7
0	15	10	3	2

* Supplied by Standard Brands, Incorporated.

tered orally. Because the results after 5 days were inconclusive, supplementation was continued. The assays were finally terminated after 7 weeks. The results are given in Table I. In this table weekly fluctuations in wrist stiffness are disregarded; only the over-all changes in stiffness which are manifest at the end of the 7-week period are indicated.

Prophylactic tests also were carried out with ergostanyl acetate on guinea pigs showing no stiffness and weighing 250-300 g at the start of the experiment. Our supply of the natural antistiffness factor was insufficient to permit its inclusion in this series. The results are shown in Table II. Some animals at all levels of ergostanyl acetate administration progressed through the whole range of wrist stiffness and, at the close of the eight-week experiment, had perfectly rigid wrists under the conditions of the test (1+ stiffness).

Summary and conclusions. Wrist stiffness was produced in guinea pigs by feeding two different commercial, pelleted rations. Both in therapeutic and in prophylactic tests, however, the untreated controls fared as well as the animals which received reputedly active supplements in amounts which have previously been shown to be more than adequate to give positive responses.^{1,6} We interpret these results to indicate that (1) the mere production of wrist stiffness in guinea pigs does not necessarily yield animals satisfactory for assay purposes, that (2) the commercial, pelleted diets used by us and by Oleson *et al.*¹ are unsuitable as basal rations for the routine assay of the Wulzen antistiffness factor, and that (3) the activities of the steroids tested by Oleson *et al.* should be redetermined with animals depleted on more satisfactory basal rations.