the shifts in PR ratio could have been the result of an infection rather than of polyarthritis, *per se.*

The animals treated with exudate plus cortisol were in much better physical condition than the animals treated with exudate alone as evidenced by appearance of the coat, activity of the animals, and mortality figures (Table I). This would indicate that under the conditions of these experiments the adrenal replacement (or anti-stress) effects of cortisol and the anti-inflammatory effects of cortisol were dominant over the pro-infective effects.

In subsequent experiments, using new batches of MRLS exudate, we were unable to reproduce the expected incidence of polyarthritis. If the arthritogenic factor in the exudate used earlier was an organism growing in the tumor as Jasmin's work suggests, perhaps we lost our "culture" during serial transplantations of the tumor.

Microscopic examination of the joints after 5 days suggested that the inflammatory reactions originated in the periarticular tissues. Conclusive proof of the site of origin will require sectioning the joints at earlier stages of the reaction and perhaps examination of serial sections. Regardless of whether the lesions originate in the periarticular or the synovial tissues, the severe inflammatory reaction produced by MRLS exudate offers a promising approach to study of inflammation and anti-inflammatory drugs.

Summary. A single injection of exudate from Murphy rat lymphosarcoma produced polyarthritis and elevated polysaccharide/protein ratios of the serum. In a later series of experiments the incidence of arthritis was much less. When arthritis was produced cortisol inhibited the incidence and severity of the arthritic lesions and partially reversed the shift in polysaccharide/protein ratio. Phenylbutazone had no consistent effect on polyarthritis or serum ratios.

1. Jasmin, G., *Rev. Can. de Biol.,* 1956, v15, 107.
2. ———, *Ann. Rheum. Dis.,* 1957, v16, 365.
3. Jasmin, G., Bois, P., Selye, H., *ibid.,* 1956, v15, 269.
4. Stidworthy, G., Payne, R. W., Shetlar, C. L., Shetlar, M. R., *J. Clin. Invest.,* 1957, v36, 309.
5. Shetlar, M. R., Foster, J. V., Everett, M. R., PROC. SOC. EXP. BIOL. & MED., 1948, v67, 125.
6. Payne, R. W., Shetlar, M. R., Bullock, J. A., Patrick, D. R., Hellbaum, A. A., Ishmael, W. K., *Ann. Int. Med.,* 1954, v41, 775.
7. Brodie, B. B., *J. Pharm. & Pharmacol.,* 1956, v8, 1.
8. Roberts, S., *J. Biol. Chem.,* 1953, v200, 77.
9. Winzler, R. J., *Determination of Serum Glycoproteins,* in D. Glick: *Methods of Biochemical Analysis,* 1955, Interscience Publ., New York, v2, 279.
10. Wöhler, F., Ghiassi, K., *Arch. Exp. Path. u. Pharmakol.,* 1957, v230, 161.

Received June 17, 1958. P.S.E.B.M., 1958, v99.

Response to Exogenous Gonadotrophins in Absence of Dietary Protein.* (24246)

HERBERT H. SREBNIK, MARJORIE M. NELSON AND MIRIAM E. SIMPSON
Institute of Experimental Biology and Department of Anatomy, University of California, Berkeley

Rats on low-protein or protein-free diets exhibit marked disturbances of the reproductive system as shown by prolonged anestrus, ovarian and accessory organ atrophy and by failure to maintain pregnancy(1,2). Estrone and progesterone at dosage levels effective in rats hypophysectomized and ovariectomized soon after breeding(3) successfully maintained pregnancy in the absence of dietary

* Aided by grants from U.S.P.H.S. We are greatly indebted to Dr. Randolph Major, Merck and Co., Rahway, N. J., for generous supplies of 2-methyl-1,4-napthoquinone and crystalline B vitamins; to Dr. Elmer L. Severinghaus, Hoffmann-LaRoche, Nutley, N. J. for crystalline *d*-biotin, *d*-calcium pantothenate, and *dl*-alpha-tocopherol; and to Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y. for synthetic pteroylglutamic acid.

protein(4). The gonadal atrophy resulting from such diets suggested a reduction in circulating gonadotrophic hormones or an impairment of ovarian sensitivity. This study was designed to test the response of female rats to administration of various gonadotrophins in the complete absence of dietary protein.

Methods. Female rats (Long-Evans strain) approximately 80 days of age and 200 g in body weight were fed a purified diet, free of protein, but containing all other known dietary essentials.† Vaginal smears were examined daily, and the animals weighed every 5 days. After 5 weeks on the protein-free diet, the rats were distributed into equivalent groups of 3 to 5 rats according to body weight and length of anestrus. When injection of gonadotrophins was begun the rats had been in anestrus for approximately a month and had lost one-third of their initial body weight. Four gonadotrophic hormones‡ were injected: pituitary follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH), human chorionic gonadotrophin (HCG) and equine gonadotrophin (PMS). Varying levels of FSH, ICSH and HCG were given subcutaneously once daily for 4 successive days; PMS was administered in a single subcutaneous dose. As a standard of reference for response to hypophyseal gonadotrophins, hypophysectomized rats, 17-20 days post-operative, were used. These rats were similar in age and body weight to the protein-deficient animals and were maintained on a stock diet of natural foodstuffs.§ In experiments involving non-pituitary gonadotrophins normal immature rats,‖ 24 days of age, were injected parallel with protein-deficient animals. Hypophysectomized rats are less responsive to low levels of HCG and PMS(6) than intact animals and, therefore, are not suitable as controls for these gonadotrophins. All rats were autopsied 96 hours after onset of injection. Uteri were freed of their oviducts, drained of fluid and weighed. Ovaries were weighed, fixed in Bouins solution and stained with hematoxylin and eosin for histological study. Criteria for ovarian stimulation were those described by Evans *et al.*(7). Follicle stimulating activity was determined by resumption of follicular growth, and interstitial cell stimulating activity by repair of the atrophic interstitial cells, *i.e.*, return to normal nuclear pattern and cell size. Production of fully mature follicles and of corpora lutea as well as uterine response gave additional evidence of ovarian sensitivity. The critical dosage levels for minimal response to each gonadotrophin were confirmed at least once.

Results. Follicular development was about equally retarded in the ovaries of uninjected protein-deficient and of adult hypophysectomized rats, both containing only small follicles. Follicular development was perhaps slightly greater in protein-deficient rats, beginning antrum formation being observed occasionally. Persistent corpora lutea were present in the ovaries of both types of rats. The interstitial cells in the protein-deficient animals had the same morphological and staining characteristics as in hypophysectomized rats ("wheel cells"), though they appeared to be increased in number.

Pituitary *FSH* had no effect on follicular growth in protein-deficient or in adult hypo-

† The protein-free diet consisted of sucrose 88%, hydrogenated cottonseed oil 8%, salts No. 4(5) 4%. Crystalline vitamins/kg of diet were: vit. B_{12} 0.05 mg, *d*-biotin 0.6 mg, 2-methyl 1,4-naphthoquinone 10 mg, thiamine HCl 10 mg, pteroylglutamic acid 11 mg, riboflavin 20 mg, p-aminobenzoic acid 20 mg, niacin 40 mg, d-calcium pantothenate 100 mg, inositol 800 mg, choline chloride 1000 mg. All rats received weekly a fat-soluble vitamin supplement of 800 USP units vit. A, 115 chick units vit. D, 6 mg synthetic alphatocopherol and 650 mg corn oil (Mazola).

‡ FSH prepared from sheep pituitaries and ICSH prepared from beef pituitaries by ammonium sulfate fractionation; HCG ("Antophysin"), prepared by Winthrop Laboratories; PMS ("Gonadin"), prepared by Cutter Laboratories.

§ Diet I is composed of wheat 67.5%, casein 15%, skim milk powder 7.5%, hydrogenated vegetable oil 6.75%, fish oil 1%, iodized NaCl 0.75%, $CaCO_3$ 1.5%. This was supplemented every day by a wet mash of the same diet.

‖ Normal immature rats received the regular stock diet (Diet XIV) consisting of wheat 68.5%, casein 5%, fish meal 10%, alfalfa leaf meal 10%, fish oil 5%, iodized NaCl 1.5%. Lettuce was given 2-3 times weekly.

TABLE I. Response to Hypophyseal FSH in Protein-Deficient and in Hypophysectomized Adult Rats.

Total dose, RU*	No. of rats	Ovaries: Wt, mg		Ovaries: Follicles†	Ovaries: Interstitial cells	Uterine wt, mg	
		Protein-deficient					
0	11	25	2‡	s	Atrophic	80	4‡
1	3	26	5	s	"	77	8
2	8	30	4	sm	"	84	4
4	11	34	2	m	"	94	8
8	8	44	3	m–l	"	167	18
		Hypophysectomized					
0	11	29	2	s	Atrophic	68	3
1	9	29	3	s	"	68	4
2	9	35	3	sm	"	81	10
4	9	44	2	m	"	95	13
8	9	54	5	m–l	"	170	12

* 1 RU (rat unit), as defined by Evans *et al.*(7), represents minimal total amount which, inj. subcut. over a 3-day period, caused resumption of follicular growth in rats hypophysectomized at 26-28 days of age and 6-8 days post-operative.

† Follicles were measured with ocular micrometer and designated as small (s) = 375 μ, small medium (sm) = 450-500 μ, medium (m) = 550-650 μ, medium-large (ml) = 700-750 μ, and large (l) = 800-1000 μ.

‡ ± stand. error of mean.

physectomized rats when 1 rat unit (RU) was given (Table I). Both groups responded, however, to 2 RU with a slight increase in follicle size and beginning antrum formation. With the highest dosage, 8 RU, uterine weights were increased to the same extent. No effect on the atrophic interstitial tissue was observed in either type of rat up to the 8 RU dose level. Injection of ½ RU of pituitary *ICSH* in protein-deficient rats (Table II) caused a detectable increase in the amount of interstitial cell cytoplasm and in the intensity of the staining reaction (designated as "partial repair"). Further increase in cell size and staining intensity, as well as increase in nuclear size and some scattering of the clumped chromatin, were observed with 1 RU ICSH. Hypophysectomized rats required twice as much ICSH, namely 1 RU and 2 RU respectively, for similar responses.

HCG resulted in follicular growth and increased uterine weights in protein-deficient rats at 1.25 IU (Table III); 2.5 IU HCG induced corpus luteum formation and partial repair of interstitial tissue. In normal immature rats, used in comparisons of response to

TABLE II. Response to Hypophyseal ICSH in Protein-Deficient and in Hypophysectomized Adult Rats.

Total dose, RU*	No. of rats	Ovaries: Wt, mg		Ovaries: Follicles	Ovaries: Interstitial cells	Uterine wt, mg	
		Protein-deficient					
0	24	25	1†	s	Atrophic	79	3†
.25	16	30	2	"	"	95	5
.5	16	29	1	"	Partial repair	86	3
1.0	16	28	1	"	*Idem*	88	2
		Hypophysectomized					
0	16	31	2	s	Atrophic	72	4
.5	7	29	1	"	"	84	5
1.0	15	29	2	"	Partial repair	88	5
2.0	16	32	2	"	*Idem*	87	3

* 1 RU (rat unit), as defined by Evans *et al.*(7), represents minimal total amount which, inj. intraper. over a 3-day period, caused repair of "deficient" interstitial cells in rats hypophysectomized at 26-28 days of age and 6-8 days post-operative. When ICSH is inj. subcut., as in this study, approximately 4 times as much of the hormone is required.

† ± stand. error of mean.

TABLE III. Response to HCG in Protein-Deficient and in Normal Immature Rats.

Total dose, IU	No. of rats	Ovaries: Wt, mg		Ovaries: Follicles	Ovaries: Interstitial cells	Uterine wt, mg	
		Protein-deficient					
0	11	26	2†	s	Atrophic	83	4†
.3–.6	6	26	1	s	"	108	18
1.25	8	29	2	sm, m	"	185	20
2.5	8	38	3	m–ml, CL*	Partial repair	185	10
3.75–5.0	6	41	3	m–l, CL	Repair	164	9
		Normal immature					
0	10	19	1	sm, few m	Epithelioid	22	1
.6	10	19	1	*Idem*	"	28	2
1.2	10	18	2	"	"	23	2
2.5	10	18	1	ml, few l	"	116	18
5.0	5	28	3	few ml, CL	"	102	6

* Newly formed corpora lutea. † ± stand. error of mean.

this gonadotrophin, 1.25 IU caused no follicular development, 2.5 IU being required for follicular and uterine growth and 5 IU for corpus luteum formation. Only 0.25 IU of *PMS* was needed to stimulate follicular development and increase uterine weights in protein-deficient rats (Table IV), and 0.5 IU resulted in corpus luteum formation and partial repair of the interstitial tissue. In normal immature rats higher levels of PMS, namely 0.5 - 1 IU, were necessary for follicular enlargement and uterine response, and 1 IU for corpus luteum formation.

Discussion. It has been shown in this study that the ovaries of protein-deficient rats responded to exogenous gonadotrophins. Pituitary FSH and ICSH were at least as effective as in hypophysectomized rats of similar age and body weight, whereas HCG and PMS were twice as effective as in normal immature rats. Starvation or food restriction, conditions in which protein consumption is correspondingly reduced, did not interfere with the response of the ovaries to pituitary or non-pituitary gonadotrophins (*e.g.* 8,9,10,11). Similar results were obtained in lysine deficiency(12). The effects of small amounts of hormone, or of graded doses, however, have not been adequately explored; moreover, no purified hypophyseal gonadotrophins have been tested during inanition and their effects compared with those in hypophysectomized animals.

Ovarian responsiveness to gonadotrophins, under conditions comparable to those used here, has been investigated during Vit. B_6 deficiency(13). B_6-deficient rats required 8 times as much FSH and twice as much ICSH for follicular stimulation and interstitial repair as hypophysectomized rats of comparable age and body weight. The FSH and ICSH preparations were identical with those

TABLE IV. Response to PMS in Protein-Deficient and in Normal Immature Rats.

Total dose, IU	No. of rats	Ovaries: Wt, mg		Ovaries: Follicles	Ovaries: Interstitial cells	Uterine wt, mg	
		Protein-deficient					
0	14	25	2*	s	Atrophic	89	2*
.1	9	28	2	s	"	101	7
.25	9	29	2	sm	"	188	19
.5	14	37	2	m–l, CL	Partial repair	204	12
1.0	10	42	3	m–l, CL	Repair	160	12
		Normal immature					
0	19	17	1	sm, few m	Epithelioid	27	2
.5	14	16	1	sm, few m–ml	"	63	12
1.0	15	25	1	few m, ml, CL	"	110	3

* ± stand. error of mean.

used in this study. It would appear, therefore, that loss of ovarian function in B_6 deficiency may be partly due to impaired ovarian sensitivity. In protein-deficient rats, where no such impairment exists, reduction of circulating gonadotrophins is a more likely explanation. Lack of circulating gonadotrophins has previously been suggested as an explanation for the gonadal atrophy resulting from inanition(8,9,10).

The reason for the greater effectiveness of HCG and PMS in protein-deficient rats than in normal immature rats is not clear. These hormones may be more rapidly inactivated or excreted in normal immature rats, or the ovaries of sexually immature animals may be refractory to low levels of these gonadotrophins. Both HCG and PMS exert gonadotrophic action at lower doses in normal than in hypophysectomized rats(6). It is commonly assumed that these hormones stimulate the pituitary in the normal rat, the FSH released acting synergically with the ICSH-like component of the injected hormone. Follicular growth following injection of HCG and PMS into protein-deficient rats might then result from a similar mechanism. The pituitaries of protein-deficient rats have already been shown by bioassay to contain FSH (14), and this may be released under the stimulus of these hormones.

Summary. Adult female rats were given a protein-free diet for 5 weeks and their response to graded amounts of gonadotrophic hormones determined. Protein-deficient rats responded to FSH with follicular growth at the same dosage levels as adult hypophysectomized rats of similar age and body weight. ICSH, as judged by stimulation of interstitial tissue, was slightly more effective in protein-deficient than in hypophysectomized rats. HCG and PMS induced follicular growth, corpus luteum formation and increased uterine weights in protein-deficient rats at one-half the dosages necessary in normal immature rats.

1. Guilbert, H. R., Goss, H., *J. Nutrition,* 1932, v5, 251.

2. Nelson, M. M., Evans, H. M., *ibid.,* 1953, v51, 71.

3. Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 65.

4. Nelson, M. M., Evans, H. M., *Endocrinol.,* 1954, v55, 543.

5. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.,* 1941, v138, 460.

6. Fraenkel-Conrat, H., Li, C. H., Simpson, M. E., Evans, H. M., *Endocrinol.,* 1940, v27, 793.

7. Evans, H. M., Simpson, M. E., Tolksdorf, S., Jensen, H., *ibid.,* 1939, v25, 529.

8. Mulinos, M. G., Pomerantz, L., Smelser, J., Kurzrok, R., PROC. SOC. EXP. BIOL. & MED., 1939, v40, 79.

9. Werner, S. C., *ibid.,* 1939, v41, 101.

10. Maddock, W. O., Heller, C. G., *ibid.,* 1947, v66, 595.

11. Rinaldini, L. M., *J. Endocrinol.,* 1949, v6, 54.

12. Courrier, R., Raynaud, R., *Comptes rend. Soc. Biol.,* 1932, v109, 881.

13. Wooten, E., Nelson, M. M., Simpson, M. E., Evans, H. M., *Endocrinol.,* in press.

14. Srebnik, H. H., Nelson, M. M., *Anat. Rec.,* 1957, v127, 372.

Received June 17, 1958. P.S.E.B.M., 1958, v99.

Effect of Pre-Feeding of Fat on I^{131} Triolein Absorption in Subtotal Gastrectomy Patients. (24247)

MALCOLM P. TYOR AND JULIAN M. RUFFIN
(Introduced by E. A. Stead)
Departments of Medicine, Duke University Medical Center, Veterans Administration Hospital, Durham

Oral administration of I^{131} labeled triolein to patients with subtotal gastrectomy (Billroth II) frequently yields abnormal results consisting of reduced levels of blood radioac-

* Work supported in part by U. S. Public Health Grant A1987.