TABLE II.
Effect on Body Weight, Food Intake, and Liver Fat of Adding Methionine to a Diet Free of Sulfur-Containing Amino Acids.

Group	Treatment	No. of rats	Wt of animals: Initial, g	Wt of animals: Change, %	Food intake, g/day	Liver: Wt, g	Liver: Total lipid, %
I	Basal diet	15	160 (149-172)	29.8 ± 0.8	5.5 ± 0.2	5.30 ± 0.24	19.7 ± 1.7
II	Basal diet + Methionine	15	164 (146-182)	−21.0 ± 0.8	6.3 ± 0.2	5.50 ± 0.14	17.9 ± 1.4

the amount of methionine required to show a lipotropic effect may be considerably increased in a diet containing minimal amounts of choline. In the present investigation crystalline vitamin B components were used, while in those quoted above[3,5] the animals were fed 0.5 g of yeast daily. This amount of yeast contains more than 1.0 mg of choline[7] which is an appreciable percentage of the daily requirement. It is possible, also, that some other component of yeast works in conjunction with methionine.

Summary. Under the experimental conditions chosen, methionine at a level of 50 mg per day (0.8% of the diet) did not prevent the development of fatty livers in rats fed a diet in which a mixture of essential amino acids replaced protein.

[7] Fletcher, J. P., Best, C. H., and Solandt, O. M., *Biochem. J.*, 1935, **29**, 2278.

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Implantation of Riboflavin Pellets in Animals and Man.

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Nutritional studies of the Palestinian population have shown that riboflavin deficiency is very often observed in this country,[1] and that its incidence is far more frequent than that of other vitamin deficiencies.[2] The clinical manifestations of this deficiency, such as glossitis, cheilosis, corneal vasicularization, etc., were particularly common in pregnant women. Low riboflavin excretion in the urine (averaging 95 γ per liter instead of 360 γ per liter in normal pregnant women) was found in 190 (21%) out of 900 pregnant women studied.[3] Furthermore, observations have been reported as to the harmful effect of riboflavin deficiency on the course of pregnancy (prematurity), on the condition of the fetus *in utero* (higher incidence of antenatal death) and upon the postpartum period (agalactia and hypogalactia).[4]

Surprisingly enough, these clinical manifestations appeared not only in the poorer classes, in which riboflavin intake was inadequate, but also in well nourished subjects. The fairly frequent incidence of riboflavin deficiency in well nourished individuals is principally due to inadequate resorption of this vitamin. The frequent occurrence of various intes-

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[2] Berger-Rabinowitz, S., Doctor's Dissertation, 1945, Hebrew University, Jerusalem.

[3] Braun, K., Bromberg, Y. M., and Brzezinski, A., *J. Obst. and Gyn., Brit. Empire*, 1945, **52**, 1.

[4] Brzezinski, A., Bromberg, Y. M., and Braun, K., *J. Obst. and Brit. Emp.*, 1947, in press.

tinal and hepatic diseases (amebic dysentery, gastric achlorhydria and hypochlorhydria) and various forms of chronic hepatitis is undoubtedly the most important etiologic factor in the production of deficiency symptoms in these subjects. Riboflavin therapy generally gives striking results in those cases in which the deficiency is induced by insufficient intake.[1,3] On the other hand, poor results are generally observed when riboflavin is administered to subjects suffering from riboflavin deficiency due to inadequate resorption. In such patients the oral administration of riboflavin seems to be ineffective. Furthermore, riboflavin administered parenterally or intravenously, is eliminated from the body very rapidly. Thus, in order to maintain a constant level in the tissues and to prevent the rapid elimination of riboflavin,[3,4] the daily dose must be divided into many subdoses.

The poor therapeutic results obtained with riboflavin in patients suffering from diseases of the digestive tract led us to attempt the implantation of riboflavin pellets so as to afford adequate and continuous resorption of this vitamin. This method of supply seems to us particularly advantageous in patients who require repeated daily injections of riboflavin for a relatively long period. In addition, the difficulties involved in handling this substance which is easily inactivated when exposed to light, and the unreliability and lack of cooperation of patients, induced us to search means of supplying riboflavin in controlled quantities.

Technic. Pellets implanted in rats. Pellets containing riboflavin* adsorbed on Fuller's earth were used at first but later discarded, because they were completely absorbed in the body within a fortnight. Much better results were obtained when a mixture of 20 mg cholesterol plus 20 mg riboflavin was used. Pellets prepared in this way lost 90% of their riboflavin content within 1-1½ months. In the present report the results obtained with this type of pellet will be described. It seems that better results can be obtained with pellets prepared by melting together cholesterol with riboflavin. Results with this method will be described elsewhere. Sterilization of the pellets was carried out by heating them twice for 4 hours at 80°C.

Pellets implanted in patients (humans). The pellets implanted in patients were made by mixing 50 mg cholesterol with 50 mg riboflavin. This mixture was made up into 4 pellets. They were sterilized by heating them 3 times for 4 hours at 80°C. The riboflavin was handled in darkness; implantation was carried out subcutaneously in the lateral aspect of the thigh. Four pellets containing 50 mg riboflavin in all were implanted into each patient.

Riboflavin determination in the urine and the recovered pellets was carried out by the microbiological method of Snell and Strong.[5,†]

Results. Resorption of riboflavin pellets in rats. A sterile pellet containing 20 mg of riboflavin with 20 mg of cholesterol was implanted in the medial muscles of the thigh of 5 female albino rats weighing 150 g each. The rats were kept on a stock diet consisting of sprouted wheat, oats and bran supplemented by milk and vegetables in season. Riboflavin excretion in the urine was determined at intervals of 2 days for 2 weeks preceding the implantation. After the implantation riboflavin excretion was determined every other day, and when the riboflavin level had returned to normal, at weekly intervals. When the rats were sacrificed, the "ghost" of the pellet was recovered and its riboflavin content determined. It contained on an average 20% of the original riboflavin content one month after implantation. The results are compiled in Table I. Table I demonstrates that increased riboflavin excretion persisted for one month after implantation. Later on, riboflavin excretion returned to the normal level, thus proving the discontinuance of any further resorption of riboflavin from the pellet.

Resorption of riboflavin pellets in human patients. At first one normal well nourished

* We are indebted to "Assia" Chem. Lab., Tel-Aviv, for a generous supply of riboflavin.

5 Snell, E. E., and Strong, F. M., *Ind. and Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

† We are indebted to Dr. Lea Bichowsky for these analyses.

TABLE I.

Riboflavin Excretion in the Urine of 5 Adult Female Albino Rats Before and After Intramuscular Implantation of Pellets Containing 20 mg Riboflavin (with 20 mg Cholesterol). The figures are for gamma of riboflavin per liter urine.

Days before implantation	Rat No. 1	Rat No. 2	Rat No. 3	Rat No. 4	Rat No. 5
14	115	90	95	100	90
12	120	150	100	110	100
10	110	170	110	90	95
8	90	140	90	120	110
6	100	100	75	80	115
4	115	70	90	90	110
2	95	130	110	100	95
Days after implantation					
2	500	500	530	800	600
4	360	300	960	600	700
6	290	290	510	500	860
8	240	250	450	420	510
10	500	300	550	640	400
12	600	900	300	410	350
14	400	800	420	640	280
18	600	480	450	800	700
21	650	320	400	700	840
28	600	200	200	690	600
35	125	260	140	400	510
42		225	110	120	90
49		280		110	
56		170		90	
63		80		100	

woman was implanted with riboflavin pellets (4 × 25 mg) containing 50 mg riboflavin in all. The riboflavin excretion in a 24-hour specimen of urine was determined (Table II) at 2-day intervals for a period of 2 weeks before the implantation and was found to vary between 650 γ-1000 γ. The patient was kept on a normal diet which would have allowed for variations in the daily riboflavin excretion, between 250 γ-1000 γ per liter of urine. A distinct increase in the amount of riboflavin (1200 γ-2800 γ) excreted in this woman's urine persisted for approximately 1½ months following implantation. This result led us to implant riboflavin in a patient suffering from characteristic glossitis, seborrheic facial lesions, and ocular manifestations such as corneal vascularization. Previous treatment of this patient with orally administered riboflavin in daily doses of 20-30 mg was without any effect. These poor therapeutic results were explained by the existence of complete gastric achlorhydria and chronic diarrhea due to amebiasis. Parenteral riboflavin treatment consisting of 5 mg injections thrice daily gave moderately good but temporary results since 2 days after discontinuance of the treatment signs of riboflavin deficiency reappeared. Implantation of pellets containing 50 mg of riboflavin gave good results in this patient with complete absence

TABLE II.

Riboflavin Excretion in the Urine of a Normal Female Patient (A) and One Pregnant Patient (B) Suffering from Riboflavin Deficiency Before and After Intramuscular Implantation of Pellets Containing 50 mg Riboflavin (with 50 mg Cholesterol). The figures are for gamma of riboflavin per liter urine.

Days before implantation	Patient A	Patient B
14	870	100
12	900	150
10	650	120
8	800	200
6	950	250
4	1000	150
2	780	170
Days after implantation		
2	1200	1300
4	1800	1500
6	2000	1500
8	2400	2500
10	2800	3000
12	2000	3500
14	2600	3000
18	2200	3500
21	2500	3200
28	2000	2000
35	2000	1500
42	1500	1000
49	950	850
56	690	500

of deficiency symptoms for 2 months. At the same time the daily urinary excretion of riboflavin increased from the preimplantation level of 100 γ-250 γ to a concentration of 1000 γ-3500 γ per liter (Table II).

Discussion. The fact that it is possible to maintain a high riboflavin level within the body of animals and man, and to eliminate deficiency symptoms for a period of 1-1½ months, is encouraging. Cases are frequently encountered where regular oral intake of the required riboflavin is ineffective because of inadequate resorption in patients with gastric, intestinal or hepatic diseases. In such patients the riboflavin is only utilized when injected[6] in repeated daily doses. Since the maintenance of such treatment is often difficult, if not impossible, we believe that the implantation of pellets has a definite practical value. Furthermore, we intend to apply this method to other vitamins.

Summary. 1. A method is described for the preparation of riboflavin pellets that can be implanted in animals and in humans.

2. The effect of these riboflavin pellets (containing cholesterol) in cases of retarded riboflavin resorption lasts from 1-1½ months.

3. The riboflavin pellets implanted in a patient suffering from riboflavin deficiency brought about complete relief of relatively long duration from deficiency symptoms.

4. The method may be of practical value for patients suffering from riboflavin deficiency due to inadequate resorption, and when repeated injections are difficult to carry out.

[6] Bicknell, F., and Prescott, F., *The Vitamins in Medicine*, London, W. Heinemann, Ltd., 2nd edit., 1946.

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Action of Sodium Salicylate on Hyaluronidase.

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The purpose of this communication is to report certain experiments on the inhibition of hyaluronidase by sodium salicylate *in vivo* and *in vitro*.

Despite wide therapeutic usefulness little is known concerning the mechanism of action of salicylates. A number of investigators have shown that sodium salicylate at relatively high concentrations (0.05 *M*-0.5 *M*), causes a reversible denaturation of certain biologically active proteins.[1-4] Euler and coworkers[5] found that salicylates inhibit certain enzyme reactions in concentrations as low as 0.01 *M*. Some of these are partially reversible by diphosphopyridine nucleotide. Ivanovics[6] observed that the growth of certain organisms such as *Staphylococcus aureus* was inhibited by concentrations as low as 0.002 *M* while others such as *Proteus morganii* required much larger amounts. He correlated this difference with the ability to synthesize pantothenic acid and found that added pantothenic acid or to a lesser extent β,β-dimethyl-α-hydroxy-γ-butyrolactone was able partially to reverse the salicylate inhibition of *S. aureus*. He concluded that sodium salicylate owes its antibacterial effects to 2 different properties, namely specific inhibition of the synthesis of β,β-dimethyl-α-hydroxy-γ-butyrolactone and nonspecific protein denaturation.

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[2] Holden, H. F., *Austral. J. Exp. Biol. Med.*, 1937, **15**, 43.

[3] Bawden, F. C., and Pirie, N. W., *Biochem. J.*, 1940, **34**, 1278.

[4] Best, R. J., *Austral. J. Exp. Biol. Med.*, 1946, **24**, 27.

[5] Euler, H. v. and Ahlstrom, L., *Z. physiol. Chem.*, 1943, **279**, 175.

[6] Ivanovics, G., *Z. physiol. Chem.*, 1942, **276**, 33.