old that had not been used before were employed for all the points except .125 and .25 gamma. These were obtained 3 weeks later on 6 and 11 capons respectively. These 2 groups were so made up from the birds previously used only once that they should be equal to an average of the entire group. To find out if we were safe in combining data from "fresh" capons with those from birds previously treated, 11 of the capons selected as above were run in the second experiment on .5 gamma; these 11 gave a response identical with the first. (See Table).

From Fig. 2 it is seen that the curve is logarithmic from .125 to 4 gamma. The best fit is from .25 to 2 gamma.

Fig. 3 compares the curve in Fig. 1 with a curve obtained by injection. The latter curve is obtained from Greenwood, *et al.*[8] For comparison, the average response obtained from the injection of 100 gamma androsterone into 15 of our "virgin" capons is included.

10683

Chemical Composition and Vitamin Content of Royal Jelly.*

R. M. MELAMPY AND D. BREESE JONES.

From the Bureau of Entomology and Plant Quarantine, Department of Zoology, Louisiana State University, and the Bureau of Chemistry and Soils, United States Department of Agriculture.

In the colony of the honeybee (*Apis mellifera* L.) there are two castes of females, the queen, whose function is limited to reproduction, and the workers, who carry on all the other activities of the colony. The physiological process by which one female larva develops into a worker and another becomes a queen is assumed to be determined by its diet. For the first 2 days after hatching all female larvae apparently receive the same diet and the physiology of their development is similar. This diet is royal jelly, a secretion of the pharyngeal glands of the workers, and it is fed to the larvae at fre-

extracts of bull and ram urine and presumably of extracts of other materials or fractions containing relatively large amounts of extraneous material. In one extract of ram urine, we were consistently unable to promote comb growth above 4.5 mm regardless of the concentration. For such materials we propose as an end point the maximum dilution that will cause unmistakable comb growth (1 to 2 mm).

[8] Greenwood, Blyth, and Callow, *Biochem. J.*, 1935, **29**, 1400.

* A contribution from the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in cooperation with the Louisiana State University, and the Bureau of Chemistry and Soils, U. S. Department of Agriculture.

quent intervals by the nurse bees. After the second day the diet of the larvae that are to become workers is altered, but the queen caste continues to receive nourishment from royal jelly.

Not only is anatomical differentiation brought about by this change in the worker's diet, but the rate of development is retarded. The imago of the worker emerges after 18 days, whereas the queen is mature in 13 days. The average weight of a female larva at the time of hatching is about 0.1 mg, and in 7 days the queen has increased her weight approximately 2,500 times and the worker about 1,400 times.

Since royal jelly is believed to be responsible for this remarkable development of the queen, a knowledge of its chemical composition and nutritional properties is important. Little work seems to have been done on this problem, however. Von Planta[1] made a chemical study of royal jelly in 1888, but apparently no one else reported any work along this line until 1922, when Aeppler[2] analyzed royal jelly. Later Elser[3] determined the chemical composition of royal jelly taken from queen cells of various ages. Within the last few years several investigators have studied the vitamin E content of royal jelly in attempts to account for the extraordinary fecundity of queen bees on this basis. The experiments reported in this paper represent a part of a study of the physiology of reproduction of the honeybee.

Method of obtaining royal jelly. To provide sufficient quantities of royal jelly for chemical analysis and biological assay, the following method was used: A queenless and broodless colony was prepared with 3 to 5 pounds of bees and an ample supply of pollen and honey. Approximately 6 hours later 60 newly hatched female larvae were placed in small wax cell cups and given to the prepared colony. Twenty-four hours later, after being fed and cared for by the nurse bees, the cells containing the larvae were removed and distributed in groups of 20 to other colonies for finishing. After 48 hours the larvae were discarded, and the royal jelly was removed from the wax cells and stored at 0°C awaiting analysis.

Chemical analysis. The moisture was determined at 60°C in a vacuum oven. The Kjeldahl method was used for the estimation of total nitrogen. The quantity of nitrogen multiplied by the conventional factor 6.25 gave the value for protein. The total lipid was determined by the Kumagawa and Suto procedure as used by Slifer.[4]

[1] Planta, A. von, *Z. f. physiol. Chem.*, 1888, **12**, 327.

[2] Aeppler, C. W., *Gleanings in Bee Culture*, 1922, **50**, 151.

[3] Elser, E., *Markische Bienen-Ztg.*, 1929, **19**, 232.

[4] Slifer, E. H., *Physiol. Zool.*, 1930, **3**, 503.

The total reducing substance, calculated as glucose, was determined by a method suggested by Dr. Michael Somogyi, Jewish Hospital, St. Louis, Mo. To each gram of royal jelly in a 50 cc centrifuge tube 10 cc of 1.2N sulfuric acid was added. The tube was closed with a rubber stopper in which was inserted a 2-foot glass tube serving as a reflux condenser. The tube and its contents were heated for 3 hours in a boiling-water bath. When the digest was cool, it was neutralized with a saturated solution of barium hydroxide, phenolphthalein being used as an indicator. The solution was diluted to 100 cc and filtered. The total reducing substance in the filtrate was determined by the dinitrosalicylic acid method of Sumner.[5] The writers believe that the total reducing substance is an index of the carbohydrate content of the royal jelly, because after fermentation with yeast the filtrates produced little, if any, color with the sugar reagent.[6] Determinations of total reducing substance were made on protein-free filtrates of royal jelly and the values were in agreement with those obtained after acid hydrolysis.

The ash determinations were made in an electric muffle furnace at 600°C. The undetermined substances were calculated by difference.

The results of the chemical analysis of 8 samples taken over a period of 6 months are presented in Table I. These data demonstrate that royal jelly taken from queen cells containing larvae between 3 and 4 days old is of remarkably constant chemical composition, and are in agreement with results obtained by Elser.[3]

Biological value of royal-jelly protein. The results presented in Table I demonstrate that royal jelly provides an abundance of tissue-building material in the form of protein, as well as energy in its carbohydrate and total lipid. The biological value of royal-jelly pro-

TABLE I.
Chemical Composition of Royal Jelly.

Sample	Moisture %	Dry matter %	Protein %	Total lipid %	Total reducing substance %	Ash %	Undetermined %
1	66.50	33.50	13.38	5.03	11.16	.88	3.05
2	66.38	33.62	12.38	5.92	12.99	.82	1.51
3	65.86	34.14	12.66	5.27	12.00	.81	3.40
4	65.55	34.45	12.16	5.51	11.61	.81	4.36
5	67.42	32.58	11.97	5.26	12.10	.82	2.43
6	66.44	33.56	11.75	5.51	12.70	.76	2.84
7	64.63	35.37	11.78	5.83	14.06	.82	2.88
8	65.60	34.40	12.63	5.38	13.27	.86	2.26
Av.	66.05	33.95	12.34	5.46	12.49	.82	2.84

[5] Sumner, J. B., *J. Biol. Chem.*, 1925, **65**, 393.

[6] Somogyi, M., *J. Biol. Chem.*, 1928, **78**, 117.

tein was determined by measuring the extent to which the protein is utilized by the standard white rat, employing the nitrogen balance method of Mitchell.[7] Four white rats were fed for 3 experimental periods of 10 days each. During the first and third periods the rats received a nitrogen-free diet, and during the second period a diet containing royal jelly was fed. Thiamin, riboflavin, and vitamin B_6 were supplied to all experimental animals by feeding daily 1 cc of 1:1 dilution of Vitab Type II concentrate.† Feces and urine were collected during the last 7 days of each period. The average endogenous and metabolic nitrogen values were determined during the periods in which the nitrogen-free diet was fed. The diets are given in Table II.

TABLE II.
Diets Used in Studying the Biological Value of Royal-Jelly Protein.

Ingredient	Nitrogen-free diet %	Royal-jelly diet %
Butter	15	15.00
Lard	12	8.81
Cod-liver oil	2	2.00
Salt mixture	4	3.41
Starch	67	4.18
Royal-jelly-starch mixture	0	66.60

For the royal-jelly diet fresh royal jelly was mixed with starch and dried at a low temperature in a vacuum oven, and the dried mixture was thoroughly pulverized before the other constituents were added. The royal jelly was incorporated in such amount as to supply a protein level of 8%.

The nitrogen balance data are presented in Table III.

The average digestion coefficient was 81%, and the average biological value of royal-jelly protein was 75%. Mitchell[8] has reported biological values for food proteins ranging from 38% for cooked navy beans to 94% for whole egg. Royal-jelly protein compares favorably with Mitchell's values for beef protein, which range from 69 to 77%.

The nutritional requirements for the growth of the honeybee and the white rat may be quite different, but it is of interest that the protein of royal jelly is adequate for the rat as shown by the data presented in Table III.

Vitamin studies with royal jelly. In addition to protein, carbo-

[7] Mitchell, H. H., *J. Biol. Chem.*, 1924, **58**, 873.

† The vitamin concentrate was kindly supplied by Vitab Corp., Emeryville, Calif.

[8] Mitchell, H. H., *J. Home Econ.*, 1927, **19**, 122.

TABLE III.

Nitrogen Balance of White Rats on a Royal-Jelly Diet for 10 Days Preceded and Succeeded by 10-Day Periods on a Nitrogen-Free Diet.

Rat	Initial wt, g	Final wt, g	Avg daily food intake, g	Avg daily N intake, mg	Avg daily fecal N, mg	Est. daily metabolic* N, mg	Avg daily food N in the feces, mg	Avg daily absorbed N, mg	Avg daily urinary N, mg	Est. daily endogenous N,† mg	Avg daily food N in urine, mg	Avg daily food N utilized, mg	Digestion coefficient,‡ %	Biological value,§ %
						Nitrogen-Free Diet.								
1	100.5	95.0	7.25		8.35	1.15			22.88	23.41				
2	123.0	119.0	5.84		11.10	1.90			27.76	22.94				
3	130.0	126.0	6.95		14.25	2.05			34.16	26.69				
8	105.0	98.0	4.73		12.65	2.67			37.76	37.20				
						Royal-Jelly Diet.								
1	97.5	107.5	6.25	83.13	14.60	7.69	6.91	76.22	43.35	20.17	23.18	53.04	82	70
2	139.0	142.0	5.18	67.86	12.10	8.91	3.19	64.67	44.00	28.10	15.90	48.77	82	75
3	164.0	174.0	7.74	101.39	20.15	16.72	3.43	97.96	64.80	38.18	26.62	71.34	80	73
8	133.0	128.5	4.48	58.69	11.10	8.65	2.45	56.24	45.20	35.03	10.17	46.07	81	82
						Nitrogen-Free Diet.								
1	144.0	137.0	3.64		4.75	1.30			22.40	15.94				
2	135.0	128.5	4.30		6.60	1.53			22.48	17.06				
3	162.0	148.0	3.45		7.80	2.26			28.64	18.48				
8	123.5	116.0	4.22		5.00	1.18			19.60	16.37				

* Calculated on the basis of 1 g of food.

† First and last groups of figures calculated on the basis of 100 g of live weight.

‡ $\frac{\text{(Food N — feces N)}}{\text{Food N}} \times 100.$

§ $\frac{\text{N intake — (fecal N — metabolic N) — (urinary N — endogenous N)}}{\text{N intake — (fecal N — metabolic N)}} \times 100.$

hydrate, fat, and inorganic elements, certain vitamins are necessary for the development of an animal. Judging from what is known of the nutritional requirements of insects, one can conclude that insects require at least growth factors belonging to the vitamin-B group.

Royal jelly was assayed for its vitamin B_1 content by using the quantitative response of the white rat, since no reliable chemical means is available for the test. A modification of the Smith rat-curative procedure[9] was used. Polyneuritis was produced in rats, and a single dose of the jelly was then administered to the animals. The response indicated that the jelly contained approximately as much vitamin B_1 as is present in whole wheat, or between 1 and 1½ international units per gram.

The vitamin A content of royal jelly was studied because there is some evidence that insects require this factor, which is essential to growth in young animals and to normal nutrition in adults.

Some preliminary tests showed that rats consumed royal jelly with avidity, and also that as much as 3 g fed daily for 3 days produced no demonstrable response in vitamin-A-deficient animals. The procedure given in the United States Pharmacopoeia XI for assaying cod liver oil was then followed, except that the number of animals was limited to 2 in each group and the jelly was fed at a level of 2 g daily. A litter of 6 animals was divided into 3 pairs. One pair received no vitamin A during the assay period and served as negative controls, another pair received daily 2 g of royal jelly, and the third pair received daily U.S.P. reference cod liver oil providing 2 units of vitamin A. The animals receiving the royal jelly failed as rapidly as did the negative controls, but the animals receiving the reference cod liver oil made a satisfactory response. It appears safe to conclude, therefore, that this sample of royal jelly was devoid of any demonstrable amount of vitamin A.

Hill and Burdett[10] claimed that vitamin E was present in royal jelly and was responsible for the fertility of the queen bee. Vitamin E is a requirement for normal reproduction by certain mammals. In the female rat lacking vitamin E, pregnancy proceeds normally until a late stage, at which time the young die and are resorbed by the maternal organism. When sufficient vitamin E is provided pregnancy and the rearing of the young follow the normal course. Sterility in the female due to lack of vitamin E is curable with a small amount of this vitamin, but in the male it is incurable.

[9] Kline, O. L., Tolle, C. D., and Nelson, E. M., *J. A. O. A. C.*, 1938, **21**, 305.

[10] Hill, L., and Burdett, E. F., *Nature*, 1932, **130**, 540.

Because of the unsatisfactory nature of the rat assays conducted by Hill and Burdett, the problem was investigated more extensively by Mason and Melampy,[11] who found that when test animals were fed royal jelly in graded amounts, from 50 to 1,000 mg daily, in no case was there sufficient vitamin E to permit the completion of gestation in rats deficient in this vitamin. The results indicate that fertility of the queen caste of bees is not dependent on vitamin E as obtained in royal jelly. Schoorl,[12] Evans, Emerson, and Eckert,[13] and Haydak and Palmer,[14] confirmed these results.

Royal jelly was analyzed for vitamin C (ascorbic acid) according to the method of Bessey and King.[15] Little, if any, of this vitamin was found to be present.

Summary. The composition and vitamin content of royal jelly, the substance responsible for the differentiation of the two castes of the female honeybee (*Apis mellifera* L.), has been determined by standard chemical methods and biological assays. Royal jelly has the following chemical composition: moisture, 66.05%; protein, 12.34%; total lipid, 5.46%; total reducing substance, 12.49%; ash, 0.82%; undetermined, 2.84%. Royal-jelly protein has an average digestion coefficient of 81% and a biological value of 75% as determined by the Mitchell method. Royal jelly proved to be a good source of vitamin B_1, containing from 1.0 to 1.5 international units per gram. It contained no demonstrable amount of vitamin A or vitamin C. It has been previously shown that royal jelly contains little, if any, vitamin E.

[11] Mason, K. E., and Melampy, R. M., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 459.

[12] Schoorl, P., *Z. Vitaminforsch.*, 1936, **5**, 246.

[13] Evans, H. M., Emerson, G. A., and Eckert, J. E., *J. Econ. Ent.*, 1937, **30**, 642.

[14] Haydak, M. H., and Palmer, L. S., *J. Econ. Ent.*, 1938, **31**, 576.

[15] Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.