The effects of caffeine on the electrical and mechanical activities of the taenia coli were also investigated. Initial increases in spike frequency and tension development were observed after application of caffeine but were not sustained: 5 min after the application of caffeine, gradual decreases in spike frequency and in tension were observed and, 10 min after, action potentials and associated contractions could be observed only by the application of depolarizing current. Conversely, spike amplitudes, which were observed during the application of depolarizing current, were increased. Irregular bursts of spike potentials observed before the application of caffeine became regular and well synchronized.

On the other hand, the tension developments which were initiated by the action potentials evoked by the depolarizing currents were decreased, regardless of the increase in the amplitude of the action potential. Thus, the partial dissociation of excitation and contraction was observed (Figure 2). These secondary effects of caffeine could be observed as long as caffeine was present, and were reversible.

These effects of caffeine were not affected by reserpinization[6] (i.p. injection of reserpine (10 mg/kg) 24 h and 12 h before performing the experiments), indicating that the inhibitory effects were not via the adrenergic inhibitory nerves.

Caffeine has dual action; first excitatory, and second inhibitory. The inhibitory action of caffeine can be explained from these results, by the assumption that caffeine affects the inward movement of Ca through the cell membrane, influencing the action potential and the contraction[7].

Zusammenfassung. Nachweis des muskelrelaxierenden (elektolischen und mechanischen) Effektes von Koffein auf den Meerschweinchendarm erst reizend und sekundär hemmend. Am K-kontrahierten Präparat führte Koffein zur Erschlaffung, die bei Erhöhung des extrazellulären Ca verändert wurde.

S. SUNANO

Department of Physiology, Sapporo Medical College, S1W17 Sapporo City (Japan), 16 March 1970.

1 J. AXELSSON and S. THESLEFF, Acta physiol. scand. *44*, 55 (1958).
2 R. HERZ and A. WEBER, Fedn Proc. *24*, 208 (1965).
3 S. SUNANO, Sapporo med. J. *33*, 227 (1968).
4 J. AXELSSON and G. HOGBERG, Acta pharmac. tox. *25*, suppl. 4, 53 (1967).
5 W. BERGER, Pflügers Arch. ges. Physiol. *277*, 570 (1963).
6 K. F. FARRELL, Nature *217*, 279 (1968).
7 S. SUNANO and E. MIYAZAKI, Experientia *24*, 364 (1968).

Effect of Ingested Gossypol on the Growth Performance of Rats

The utilization of cottonseed as a source of dietary protein is greatly limited by the presence of a naturally-occurring pigment, gossypol. This compound, in its free state, has been shown to be highly toxic to non-ruminant species when ingested at levels existing in raw cottonseed meats, in solvent-extracted cottonseed meals, and in diets supplemented with these products. There is also some indication that the daily ingestion of feeds containing very low levels of gossypol, though not producing immediate overt symptoms of toxicity, may result in the accumulation of gossypol in various organs until a critical gossypol level is reached and toxic effects appear. The finding that gossypol binds with proteins and peptides, and particularly epsilon-aminolysine groups of these substances, has suggested that these gossypol complexes in the gut are not digested and absorbed, but are eliminated in the feces. Therefore, the quality of the protein in gossypol-containing diets is presumably reduced by the binding of the gossypol with the protein. Consequently, physiological disturbances observed in animals maintained on gossypol-containing diets having minimal protein requirements are probably not the result of gossypol toxicity, but the result of dietary protein insufficiency. Therefore, in order to investigate the effects of gossypol toxicity on physiological parameters, it is necessary to utilize diets which contain high quality protein in excess of the minimal protein requirements of the animal. This paper describes the growth performance of rats maintained on diets containing high concentrations of free gossypol and high levels of quality protein.

Materials and methods. The animals used in this study were 60-day-old, male rats of the CFE strain. Each rat was housed individually under uniform conditions of light (10 h light, 14 h dark) and temperature (68–72 °F). Animals were randomly divided into 3 groups corresponding to 3 diets: diets A, B, and C. The control diet (diet A) consisted of finely-ground commercial rat chow. The experimental diets were prepared by blending the ground commercial rat chow with solvent-extracted cottonseed meal obtained by the prepress solvent-extraction procedure (diet B), and with freshly-ground, raw cottonseed meats and vitamin-free casein (diet C). The composition of each diet was determined (Table I)[1–3]. Upon commencement of the feeding study, food and water were permitted ad libitum. Animals were weighed daily and their general appearance and condition noted. After 30 days, approximately half of each group was assigned to another study, and the remaining animals continued on the diets for a total feeding interval of 60 days.

Results and discussion. The results on the growth performance of rats receiving diets A, B, and C for feeding intervals of 30 and 60 days, respectively, are presented in Table II. The mean daily weight gains, final weights, and total weight gains of those animals maintained on diets B and C were significantly less ($P < 0.05$) than those maintained on diet A (control diet). These results substantiate the report that the ingestion of gossypol by non-ruminants results in a reduction of their growth rate[4]. It should be noted that the mean daily weight gain of those animals fed diet C for 30 and 60 days is not significantly different from the mean daily weight gain of those animals fed diet B, even

1 Assoc. of Offic. Agr. Chemists, 'Offic. Meth. of Analy. of the Assoc. of Offic. Agr. Chemists', 7th edn (1950).
2 Am. Oil Chem. Soc., 'Offic. and Tent. Meth. of the Amer. Oil Chemists' Soc.', 2nd edn (1945–50).
3 E. J. CONKERTON and V. L. FRAMPTON, Arch. Biochem. Biophys. *81*, 130 (1959).
4 E. EAGLE, J. Am. Oil Chem. Soc. *37*, 40 (1960).

Table I. Composition of diets[a]

Diet	Protein	Fat	Crude Fiber	Ash	Gossypol Free	Gossypol Total	Epsilon-NH_2 lysine
A (Commercial rat chow)	25.60	4.40	5.35	7.27	0.000	0.000	1.03
B (80% commercial rat chow; 20% solvent extracted cottonseed meal)	26.00	5.97	7.16	7.17	0.048	0.184	0.56
C (85% commercial rat chow; 10% raw cottonseed; 5% vitamin-free casein)	29.00	9.32	7.39	7.97	0.083	0.136	1.84

[a] All values are expressed as percentages.

Table II. Effects of gossypol-containing diets on weight response of rats[a]

Diet	No. of animals	Feeding period (days)	Initial weight	Final weight	Total gain	Daily gain
A	15	30	198.0 ± 4.5	301 ± 8.4	104.7 ± 7.8	4.5 ± 0.3
B	17	30	188.0 ± 4.8	265.9 ± 10.4[b]	78.2 ± 9.1[b]	2.6 ± 1.2[b]
C	7	30	205.1 ± 2.5	265.7 ± 9.4[b]	60.7 ± 6.8[b]	2.0 ± 0.2[b]
A	19	60	206.4 ± 5.9	371.9 ± 17.4	165.5 ± 17.2	2.8 ± 0.4
B	17	60	189.3 ± 8.0	302.5 ± 14.1[b]	93.6 ± 11.6[b]	1.6 ± 0.2[b]
C	7	60	205.1 ± 2.5	273.9 ± 33.0[b]	68.8 ± 9.3[b]	1.1 ± 0.1[b]

[a] All weight values are means expressed in grams ± S.E. [b] $P < 0.05$ compared with diet A.

though diet C contained twice the free gossypol found in diet B. This observation can probably be attributed to the higher level of available nitrogen in diet C. For example, it has been reported that the presence of soluble proteins and amino acids in the gastrointestinal tract depresses the absorption of gossypol, probably by forming combination products with gossypol which are non-toxic in monogastric animals[5-6]. This study indicates that the growth performance of rats maintained on a diet containing a high concentration of free gossypol in the presence of a high level of quality protein is depressed. The weight depression, however, is not considered to be the consequence of dietary insufficiency. The relationship between gossypol toxicity and dietary protein as shown in this study is consistent with the finding that the toxicity of gossypol can be reduced by increasing the level of protein in the diet[7]. In addition, it has been shown that experimental diets for the study of gossypol toxicity on physiological parameters can be prepared from readily available materials, thereby eliminating the need for the expensive and time-consuming preparation of diets from elementary nutrient materials[8].

Zusammenfassung. Das Wachtum von Ratten, deren Nahrung einen reichen und freien Gossypolgehalt mit Qualitätsprotein enthielt, wurde untersucht. Die wachtumshemmende Wirkung des Gossypols konnte teilweise durch Erhöhung des Eiweissgehaltes in der Nahrung kompensiert werden.

J. N. Tone and D. R. Jensen

Department of Biological Sciences, Illinois State University, Normal (Illinois 61761, USA), 18 March 1970.

[5] L. E. Castillon and A. M. Altschul, Proc. Soc. exp. Biol. Med. *74*, 623 (1950).

[6] G. V. Odell, J. T. Cronin jr., M. M. Trant and C. M. Lyman, Fedn Proc. *23*, 503 (1964).

[7] C. A. Cabell and I. P. Earle, J. Am. Oil Chem. Soc. *33*, 416 (1956).

[8] Acknowledgment. Primary standard gossypol acetic acid for method standardization was kindly provided by Dr. G. A. Goldblatt of the Southern Utilization Research and Development Division, U.S.D.A., New Orleans (Louisiana). Raw cottonseed and solvent-extracted cottonseed meal were provided by Buckeye Cellulose Corporation of Memphis (Tennessee).