cocaine, norcocaine and pseudococaine in rat brain were 0.4, 0.6 and 0.2 h respectively; that in plasma 0.4, 0.5 and 0.2 h respectively. The brain to plasma ratios 0.25, 0.5, 1, 2, 4 h post-injection were as follows: cocaine 15.9, 11.5, 7.9, 9.5, 5; norcocaine 15.7, 10.6, 9.7, 10.9, 14; pseudococaine 2.8, 4.7, 2.4, 1.4, 1.0 respectively.

Thin layer chromatographic experiments on brain extracts (30 min) of rats injected i.v. with norcocaine provided evidence for the presence of major amounts of norcocaine (72%), benzolnorecgonine (24%) and minor amounts of norecgonine (4%). Benzoylnorecgonine has earlier been shown[6] to possess potent stimulant activity intracisternally (i.c.) in the rat and formed molecular complex[22] with Ca^{2+}. Similar experiments with pseudococaine provided evidence for the presence in brain of pseudonorcocaine (12%), pseudobenzoylnorecgonine (12%), pseudobenzoylecgonine (8%) and pseudoecgonine (minor amounts) as metabolites of pseudococaine (66%).

Our preliminary studies on the pharmacological activity of these metabolites showed that pseudoecgonine and pseudoecgonine methyl ester (1 mg kg^{-1} i.c.) showed no activity in the rat. Pseudobenzoylecgonine at this dose produced intermittent convulsions lasting approximately 15 sec and death within 10 min; lower doses 0.5 mg kg^{-1} (i.c.) produced rapid heart beat, laboured breathing, running activity, shivering, disorientation, jerking and convulsions 4 min post-injection lasting until death 30 min later. A 0.25 mg kg^{-1} dose (i.c.) produced similar effects without mortality. Pseudococaine (0.5–1 mg kg^{-1} i.c. rats) produced rapid heart beat, laboured breathing, gasping and death within 1 min and lower doses produced similar effects without mortality. Norcocaine (i.v.) was approximately 2 to 3 times more potent a stimulant as compared to cocaine.

This study demonstrates that N-demethylation and C_2-epimerization of cocaine lead to compounds which have significantly different dispositional profile in the CNS, and have more potent stimulant activity as compared to cocaine. In addition, some of the polar metabolites of norcocaine and pseudococaine, e.g. benzoylnorecgonine and pseudobenzoylecgonine, possessed potent stimulant activity intracisternally in the rat.

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On the Mechanism of Pyrogenic Action of Ricin

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Summary. The incubation of rabbit white blood cells with ricin, the toxic protein of castor oil seeds, leads to the production of endogenous pyrogens. This induction can be inhibited by the antibiotics actinomycin D or cycloheximide. The results are discussed in terms of disturbed corticosteroid- and Mg^{2+}-levels.

The authors completely agree with the suggestion that all of the known pyrogenic substances cause fever on a common basis, i.e., by the induction of the so-called 'endogenous or leukocytic pyrogen'[3–5].

According to this experimentally supported hypothesis, the pyrogens, during a relatively short period of a few hours, activate the different cells of the organism. These cells, mainly the leukocytes[6, 7], the monocytes[8], and the Kupffer's cells of the liver[5], also release endogenous pyrogens in the so-called 'early' and 'late' phases into the blood stream. During this process, the cells show different metabolic changes, e.g., increased oxygen consumption, carbon dioxyde production and increased glycolysis. This endogenously released pyrogen induces fever by acting on the central nervous system[5].

It has been experimentally demonstrated and accepted that, during the so-called 'early' phase, synthetic processes, mainly protein and RNA synthesis, take place[9]. In vitro these early processes can be inhibited by substances having an inhibitory effect on protein synthesis, for example, actinomycin-D, cycloheximide and puromycine[5, 10], while the processes of the 'late' phase are resistant to these drugs.

In recent years it has been convincingly shown that ricin has a powerful pyrogenic effect on different animals[11]. The fever caused by ricin is very high and of long duration in all species, which is similar to the fever caused by the true bacterial pyrogens[3]. Therefore it is likely that ricin also causes fever by the release of endogenous pyrogen substances. If the 'early' phase of pyrogen release of the leukocytes activated by ricin is inhibited by actinomycin-D or another drug with a similar effect, this will lend considerable support to this possibility. To investigate this, the following experiments were done.

Materials and methods. All needles, syringes and solutions were sterile and pyrogen-free. Living white blood cells were obtained under sterile conditions from the blood of healthy rabbits by heart puncture. At the time of collection, 10 IU/ml heparin was added to the blood to inhibit clotting. This quantity of heparin has no effect on the endogenous pyrogen production[12]. After 25 min of centrifugation (3000 rpm, on 4 °C), the white blood cells were aspirated away carefully with a pipette and suspended

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Table I. Endogenous pyrogen inducing effect of ricin

Treatment	Maximum elevation of body temperature (°C)	Significance
Control	0.10 ± 0.10	—
Ricin	1.10 ± 0.15	$P < 0.05$

Table II. Effect of actinomycin-D and cycloheximide on the endogenous pyrogen inducing effect of ricin

Treatment	Maximum elevation of body temperature (°C)	Significance
Control	0.15 ± 0.10	—
Ricin	1.10 ± 0.10	$P < 0.05$
Ricin + actinomycin-D	0.15 ± 0.10	$P > 0.10$
Ricin + cycloheximide	0.20 ± 0.05	$P > 0.10$

in ice-cold modified Hanks' solution (KH_2PO_4 1.568 g + NaCl 8.124 g + Dextrose 2.0 g + dest. pyrogen-free water ad 1,000.0 ml). This process was repeated twice and finally the cell number was counted using the standard Hemocytometer. The final cell suspension was made so that its content was 100×10^6 cells/ml. The cell suspension prepared as above was always made fresh before each experiment. The ricin was dissolved also in modified Hanks' solution in a concentration of 5 µg/ml. For the production of endogenous leukocytic pyrogen, 1 ml of this solution was added to the incubated suspension, which suspension always had a volume of 10 ml which gave the final concentration of ricin to be 0.5 µg/ml./10-fold dilution.

The drugs applied, namely actinomycin-D and cycloheximide, were pure preparations obtained from Sigma, USA. Their solutions as well as the ricin solution were always freshly prepared before each experiment. The actinomycin-D solution contained 50 µg/ml while the cycloheximide solution contained 25 µg/ml. During the experiments these were added to the final cell suspension so that their concentration was diluted 10 times.

Production of leukocytic pyrogen. 1 ml of the leukocytic suspension containing 100×10^6 cells, was pipetted into sterile centrifuge-tubes. 1.5 ml sterile rabbit plasma was added[9], and then ricin and/or drug solution was added in 1:1 ml volumes. Finally modified Hanks' solution was added to make the final volume 10 ml. During this filling up 10 IU/ml heparin was administered also. The test-tubes prepared as above were placed in a shaking incubator at 37°C for 12 h. At the end of the incubation, the suspension was centrifuged at 3000 rpm for 1 h at 4°C, and portions were cultured for the presence of microbiological organisms. Contaminated material was discarded, and sterile solutions were stored at 4°C until used (maximum 1–2 days).

Induction of fever. Male albino rabbits of one breed, weighing 1500–2000 g were used. During the experiment, as well as 4 h before the experiment, the animals were given neither food nor water. 30 min before the application of pyrogen, the body temperature of the animals was taken. Immediately before the pyrogen injection, the body temperature was taken again. If the difference between the 2 readings was greater than 0.15°C, the animal was disqualified. Each experiment was done on a minimum of 10 animals. No animal was used more than once in any 2 days, and after 3 experiments all animals were automatically discarded. For induction of fever, 9 ml of solutions containing leukocytic pyrogen were given i.v. After the injection, the body temperature was taken every 15 min with the help of a thermoelectric thermometer, (TRI-R, USA) having an accuracy of 0.05°C. The determination took place in the rectum, 8 cm from the anus. A rise in temperature of less than 0.20°C was not considered meaningful.

Supplementary experiments. a) Considering that theoretically it is possible that the applied drugs in some way directly inhibited the endogenous pyrogen, supplementary experiments were carried out to exclude this possibility. To a solution containing endogenous pyrogen already induced by ricin, actinomycin-D or cycloheximide was added in the same concentration as previously. After 24–48 h incubation at 4°C, no decrease was noticed on the pyrogenic effect of endogenous pyrogen, i.e., on the performed endogenous pyrogen the actinomycin-D or cycloheximide had no effect.

b) Since it is possible that the drugs employed reduced endogenous pyrogen production by killing the cells, a second series of supplementary experiments were performed. 200×10^6 cells were incubated with a proper dose of actinomycin-D or cycloheximide, for 6 h at 37°C, without ricin. At the end of the incubation period, the solution was centrifuged. The supernatant was discarded and the cells were washed twice with modified Hanks' solution. After repeated centrifugation, 100×10^6 cells were incubated with ricin, while another 100×10^6 cells were incubated with ricin, actinomycin-D and cycloheximide, respectively. This second incubation was carried out in a way similar to that used in the main experiment. Those cells which, during this second incubation, were treated with actinomycin-D or with cycloheximide alone did not produce endogenous pyrogen, while those treated with ricin only produced it without change.

c) To exclude chemical processes between ricin and actinomycin-D or cycloheximide, because of which ricin could have lost its endogenous pyrogen inducing effect, ricin and actinomycin-D, as well as ricin and cycloheximide were incubated together in the doses described above for 12 h at 37°C, without adding cells. After incubation the solution was dialyzed for 48 h against pyrogen-free distilled water. The remaining nondialyzable ricin was used for endogenous pyrogen production, in the same manner as in the main experiment. The ricin maintained its pyrogen-inducing effect which means that there no chemical reactions between ricin and actinomycin-D or cycloheximide which could interfere with the pyrogenic effect of ricin.

Results. The experimental results were analyzed statistically with the help of Student's *t*-test and are presented as follows: In the case of a typical febrile reaction, a temperature rise was noticeable with an average value of 1.00°C. The maximum of this reaction was observed after the 60th min from the time of injection. The average duration of the febrile reaction was about 3 h (Tables I and II).

Discussion. On the basis of the experiments it is clear that: 1. Ricin causes its pyrogenic effect via the activation of the so-called 'endogenous or leukocytic pyrogen'. 2. In some other experiments, BALINT[11,13] showed many other characteristic effects of ricin, which effects must

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be taken into consideration in the analysis of the possible mechanisms of the pyrogenic effect of ricin. During ricin intoxication there is an endogenous corticosteroid mobilization. The elevated blood-corticosteroid level[14] can influence the ricin-induced fever in many ways: 1. Under certain circumstances it can inhibit or retard the release of endogenous pyrogen; moreover it can produce an antipyretic effect by acting directly on the hypothalamus[15]. 2. Among the corticosteroids released by the adrenals are substances with a 5-β-hydrogen of 3-α-hydroxyl group. Steroid compounds with such a structure have a pyrogenic effect on humans[5,16]. This is a possibility which has not been investigated in this work.

BALINT[17] has shown that, under the influence of ricin, the Mg^{2+} content of blood significantly decreases, while the Ca^{2+} content increases. The physiological Ca^{2+}:Mg^{2+} ratio of the blood is thus characteristically changed.

MYERS et al.[5,18], FELDBERG et al.[19] showed almost conclusively that in warm-blooded animals the Na^+: Ca^{2+} balance of the hypothalamus is responsible for the constant body temperature. According to this statement, the shifted Ca^{2+}: Mg^{2+} ratio, as well as the the elevated Ca^{2+} level, could have an inhibitory effect on the ricin fever. This hypothesis is strengthened by BALINT's data (1965, unpublished) that ricin has no pyrogenic effect on cold-blooded animals, for example, on toads.

LIN et al.[20–22] showed convincing results to the effect that ricin antagonized the process of protein-synthesis in cells. Since it is also true that during the early production of endogenous pyrogen, protein synthesis also takes place[9], it seems very likely that the direct inhibitory effect of ricin also prevails in this situation.

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The Effect of Combined Chronic Hypoxia and N-Ethyl-N-Nitrosourea on the Carotid Bodies of Rats

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Summary. We measured the carotid body volume of rats treated with chronic hypoxia alone and chronic hypoxia together with a single neonatal injection of N-ethyl-N-nitrosourea (10 mg/kg). All the animals so treated showed enlargement of their carotid bodies, but no carotid body chemodectomas occurred.

The carotid body enlarges in man[2] and animals[3] exposed to chronic alveolar hypoxia. In the rat this is often due to a hyperplasia of the type 1 (chief) cells[4]. Neoplasms (chemodectomas) of the carotid body are rare in people living at sea-level, but their incidence is greatly increased in individuals born and permanently residing at high altitude in the Peruvian Andes[5]. Such chemodectomas may represent an extreme degree of hyperplastic response of chemoreceptor tissue to prolonged hypoxia. We investigated this problem by studying the effects of chronic hypoxia combined with the neurotropic carcinogen N-ethyl-N-nitrosourea (ENU) on the carotid body of the rat. The carotid body is of neural crest embryonic derivation[6]. We wondered if the administration of ENU would convert the hypoxic hyperplasia of the carotid body type 1 cells into neoplasia. Accordingly, we divided 110 newborn Wistar albino rats of either sex into 4 groups (Table). Group I rats were control animals and were not exposed to chronic hypoxia or given ENU. Animals in groups II and IV were given a single s.c. injection of ENU dissolved in acidified ethanol at a dose of 10 mg/kg within 24 h of birth. After weaning at approximately 21 days of age, rats in group IV were transferred to a hypobaric chamber at a pressure of 460 mm Hg (equivalent to an altitude of 4300 m above sea-level) for the remainder of their lives. Rats in Group III were not given ENU, but after weaning were transferred to a hypobaric chamber under the same conditions as rats in Group IV. When a rat died the carotid bodies were examined histologically and their volume measured by a point-counting method[7].

No significant difference was found between the mean combined carotid body volumes of the 2 groups of rats (I and II) which were not exposed to chronic hypoxia, so that it is unlikely that treatment with ENU alone can induce carotid body enlargement. Exposure to chronic hypoxia produced significant enlargement of the carotid bodies. Histologically, these enlarged carotid bodies showed the capillary dilatation and increase in volume of type 1 cells that have been described before. None of the carotid bodies had the histological appearance of chemodectoma. ENU treatment did not increase the degree of carotid body enlargement produced by chronic hypoxia. In his study of high altitude hypoxia and chemodectomas, SALDAÑA[5] described 24 carotid body neoplasms. The smallest of these was increased 10-fold as compared with the mean carotid body weight of people born and living at high altitude. None of our chronically hypoxic rats showed such a degree of enlargement of their carotid bodies. We conclude that we were unable to stimulate chemodectoma formation in any of our rats. Both the

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