ly[12]. Each sample of cortex, amygdala, hypothalamus and brain stem (pons and medulla) was pooled from 3 rats. Glutamic acid and GABA were determined after separation by paper chromatography[14]. GAD activity was measured by a radiometric procedure[15].

Results and discussion. Table I shows that glutamate increased in the cortex and amygdala of rats administered with either RbCl or CsCl as compared to animals given the same dose of NaCl. GABA levels, however, increased in the cortex, amygdala as well as hypothalamus of rats treated with RbCl. Control rats received a similar treatment using NaCl. We have previously reported that animals injected i.p. with 2 meq/kg of either 1 *M* NaCl or isotonic saline solutions as well as rats introduced i.p. with a needle (sham) showed the same values of GABA and glutamate in certain brain regions[12]. Rats administered with RbCl appeared agitated compared to those receiving the same amounts of NaCl, KCl or CsCl. The weight gain of the animals of each group was not affected by the injections of these cations. The differential distribution of glutamate and GABA as well as the enzyme GAD in the brain strongly emphasises the importance of chemical and enzymatic analyses of well defined regions of the brain. The increased glutamate in the cortex and amygdala may be related, at least in part, to the increased blood glucose level known to result from rubidium and cesium treatment[16]. Increased incorporation of ^{14}C from labeled glucose into glutamate and GABA has been demonstrated in brain perfusion studies[17]. Lithium has also been shown to increase blood glucose level[18], but it is not known whether Li^+, Rb^+ and Cs^+ share a common mechanism in producing this effect. GABA is formed in the brain from glutamate[19] and this may explain the increase in GABA levels in the cortex and amygdala where the glutamate concentration increased in rats given RbCl. GABA levels did not change, however, in brain regions showing increased glutamate produced by CsCl treatment. This did not appear to be associated with changes in GAD activity since there were none under the experimental conditions (Table II). It should be noted, however, that the lack of change in GAD levels in postassium-treated rats is probably due to the fact that the concentration of K^+ in the brain is independent of its level in plasma[20]. Of particular interest is the observation that GABA content increased significantly ($P < 0.001$) in the hypothalamus of rats treated with RbCl despite the absence of change in the glutamate level in that region. Increased GABA concentration has been associated with a decrease in neuronal excitability (for review see Ref.[21]). On the other hand, high levels of GABA were found in brains of excitable rats[22]. The latter, and also our findings showing increased GABA concentration in the hypothalamus of rats given rubidium may be a metabolic effect non-specific to this ion. Rubidium which is taken up by the brain after chronic treatment[3] produces metabolic acidosis[23] and brain excitability[1,4]. The increased CO_2, associated with high brain activity, as well as the rubidium-induced acidosis lower the tissue pH and may decrease the activity of the enzyme GABA transaminase (pH optimum 8.2) and thus result in GABA accumulation[24]. The physiological action of GABA as a vasodilator substance[24] is presumably significant in the hypothalamus; known to be intimately involved in emotional behavior[25].

Resume. L'administration chronique des cations des métaux alcalins (Na^+, Rb^+, Cs^+) à les rats a provoqué la croissance des niveaux de glutamate et GABA dans certaines régions du cerveau. On n'a remarqué aucun changement en activité GAD de ces régions.

Z. Gottesfeld, D. Samuel and Y. Icekson

Isotope and Biodynamics Departments, The Weizmann Institute of Science, Rehovot (Israel), 31 May 1972.

Table II. Glutamic acid decarboxylase (GAD) activity in brain regions of rats after chronic treatment with alkali cations

Treatment	GAD activity (μmole/g fresh wt./h) Whole brain	Cortex	Hypothalamus
NaCl	36.3 ± 1.4 (8)	30.8 ± 0.4 (6)	62.4 ± 0.6 (6)
LiCl	36.3 ± 1.0 (8)	31.9 , 31.0	63.0 , 60.2
KCl	—	31.2 ± 0.6 (3)	61.0 ± 1.7 (3)
RbCl	—	30.6 ± 0.7 (6)	62.6 ± 1.3 (6)
CsCl	—	30.9 ± 1.5 (3)	62.4 ± 2.7 (3)

Each treatment (2 meq/kg) was given i.p. twice daily for 4 days. The last injection 1 h before sacrificing rats. Means ± S.E. Number of samples in parentheses; each sample is composed of tissue pooled from 3 animals.

[14] P. Strasberg and K. A. C. Elliott, Can. J. Biochem. Physiol. *45*, 1795 (1967).
[15] E. Roberts and D. G. Simonsen, Biochem. Pharmac. *12*, 113 (1963).
[16] R. Hazard, J. R. Boissier, P. Mouillé and M. Bréteau, C.r. Séanc. Soc. Biol. *161*, 2159 (1967).
[17] G. Gombos, A. Geiger and S. Otsuki, J. Neurochem. *10*, 405 (1963).
[18] P. Plenge, E. T. Mellerup and O. J. Rafaelsen, J. Psychiat. Res. *8*, 29 (1970).
[19] E. Roberts and S. Frankel, J. biol. Chem. *190*, 505 (1951).
[20] M. W. B. Bradbury and C. R. Kleeman, Am. J. Physiol. *213*, 519 (1967).
[21] E. Roberts and E. Eidelberg, Int. Rev. Neurobiol. *2*, 279 (1960).
[22] O. Benesová, Z. Siman and K. Kunz, Physiol. Behav. *2*, 203 (1967).
[23] A. S. Relman, A. M. Roy and W. B. Schwartz, J. clin. Invest. *34*, 538 (1955).
[24] E. Roberts, K. Kuriyama and B. Haben, Adv. biochem. Psychopharmac. *2*, 158 (1970).
[25] J. V. Brady, Handk Physiol. sec. 1, *3*, 1529 (1960).

Non-Steroidal Anti-Inflammatory Drugs: Effects on the Utilization of Glucose and Production of Lactic Acid in Tissue Culture

Recently the finding that the non-steroidal anti-inflammatory drugs inhibit the production of prostaglandins E_2, and F_2 known as mediators of defensive reactions to noxious agents received much attention[1,2]. The drugs have been long recognized as multivalent substances which influence many different metabolic functions of the cell[3,4]. It has been repeatedly suggested that the effects of the drugs on the energy metabolism deserve special consideration[4-6].

In the present communication we report that various non-steroidal anti-inflammatory drugs at relatively low concentration have a profound effect on glucose utiliza-

tion and lactic acid production in cells cultured in vitro. Hence the disturbance of glycolysis may affect various biosynthetic processes including the synthesis of prostaglandins, which is as yet incompletely understood.

Primary monolayer cultures of chick embryo (CE) cells were prepared in tubes in a standard way by trypsinization. The growth medium was composed of Parker's 199 medium with 10% calf serum and antibiotics. The maintenance medium for experiments with drugs contained the same medium without added serum.

Stock solutions of aspirin and phenylbutazone (Polfa) was made in diluted NaOH. Indomethacin (Merck, Sharp and Dohme Inc.) flufenamic and mefenamic acids (Parke, Davis and Co.) derivatives of isothiazole[7] (synthetized by Dr. Z. MACHOŃ), and derivatives of barbituric acid[8] (kindly provided by Prof. B. BOBRAŃSKI) were prepared in dimethyl sulphoxide (DMSO). Further dilutions of the drugs were made in the maintenance medium. 0.1 ml of the diluted samples of the drugs were added to the 0.9 ml of the freshly exchanged medium covering 1- to 2-day-old monolayer culture of the CE cells. 3–6 cultures were used per variable. The experiments were repeated at least 3 times. The amount of glucose and lactic acid was determined in pooled tissue culture media collected after incubation for 1, 2 or 4 days at 37 °C.

Table I. The influence of various doses of indomethacin on the utilization of glucose and the production of lactic acid in the chick embryo tissue culture

Indomethacin (μmoles)	Time of incubation (days)[a]	Lactic acid[b] (μmoles)	Glucose[b] (μmoles)
none	1	1.5	5.2
none	2	1.8	4.5
none	4	3.3	3.5
125	1	1.5	5.2
125	2	2.5	4.2
125	4	5.4	2.4
250	1	2.4	5.5
250	2	4.2	4.3
250	4	6.2	2.5
500	1	4.8	3.3
500	2	6.2	2.7
500	4	8.6	1.4
1000	1	3.1	4.2
1000	2	4.1	3.3
1000	4	4.4	3.2

[a] The replicated cultures were incubated at 37° for one, two or four days. [b] Amount of the metabolites measured in the tissue culture medium after the time interval indicated.

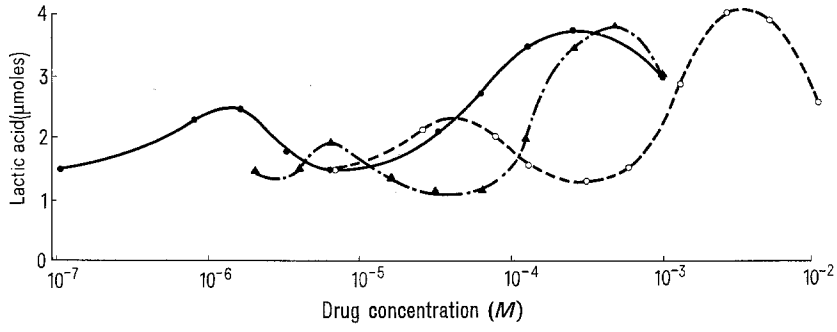
The concentration of glucose, after removing the protein with natrium wolframate, was determined by the method of NELSON[9], and lactic acid by the specific enzymatic method of HOHORST[10]. Assays for cytotoxicity of the drugs were described by INGLOT previously[3].

Results shown in the Figure illustrate the accumulation of lactic acid in tissue culture media in the presence of various concentrations of 3 drugs, after incubation for 2 days at 37 °C. It can be seen that the curves are biphasic. There is an increase of lactic acid at very low concentrations of the drugs which are completely nontoxic for the cells. At intermediate doses there is an apprently normal production of lactic acid. Then again the production of lactic acid increases and the second peak corresponds to the minimal cytotoxic doses of the drugs. The production of lactic acid is inhibited at highly toxic doses of the drugs. The character of the curves is identical for 3 drugs studied. The drugs differ only in the potential activity. Therefore, more detailed studies were performed only with indomethacin. Table I shows that the production of lactic acid is proportional to the utilization of glucose by chick embryo cells. This effect is more evident when the incubation of the drug treated cultures is prolonged from 1 to 4 days.

Similar observations were also made with other non-steroidal anti-inflammatory compounds, including some new drugs: derived of isothiazole[7] or barbituric acid[8], which were highly active (Table II). The influence of the drugs on the metabolism of glucose and lactic acid is probably not dependent upon the character of tissue. The increased production of acid metabolites was also found in secondary mouse embryo cells, L-cells, monkey kidney cells and human fibroblasts in the presence of the non-steroidal anti-inflammatory compounds (unpublished experiments).

It is conceivable that the effect described occurs not only in vitro but also in vivo. THURSTON et al.[6] found that

[1] J. R. VANE, Nature, New Biol. *231*, 232 (1971).
[2] R. FLOWER, R. GRYGLEWSKI, K. HERBACZYŃSKA-CEDRO, J. R. VANE, Nature, New Biol. *238*, 104 (1972).
[3] A. D. INGLOT, J. gen. Virol. *4*, 203 (1969).
[4] M. W. WHITEHOUSE, *Progress in Drug Research* (Birkhäuser Verlag, Basel-Stuttgart (1965), vol. 8, p. 32.
[5] J. C. G. DOERY and J. HIRSH, Experientia *27*, 533 (1971).
[6] J. H. THURSTON, P. G. POLLOCK, S. K. WARREN and E. M. JONES, J. clin. Invest. *49*, 2139 (1970).
[7] Z. MACHOŃ, Thesis Pharm. Pharmac. *21*, 325 (1969).
[8] J. GIEŁDANOWSKI and A. PELCZARSKA, Thesis Pharm. Pharmac. *23*, 325 (1971).
[9] N. NELSON, J. biol. Chem. *153*, 375 (1944).
[10] H. J. HOHORST, in *Methods of Enzymatic Analysis* (edit. Ed. H. U. Bergmeyer; Academic Press, New York 1965), p. 266.



The effect of various doses of aspirin (○---○), indomethacin (△-·-·△) and flufenamic acid (●—●) on the production of lactic acid in the chick embryo tissue cultures. All cultures were incubated for 2 days at 37 °C. In the control cultures (incubated under the same conditions but without drugs) the average amount of lactic acid was 1.5 μmoles.

Table II. Influence of various anti-inflammatory compounds on the production of lactic acid and utilization of glucose in the chick embryo culture

Compounds	Concentration (μmoles)	Lactic acid (μmoles)	Glucose (μmoles
Phenylbutazone	2500	5.5	n.d.
none	—	1.7	n.d.
Mefenamic acid	500	3.8	n.d.
none	—	2.0	n.d.
1,3-dicyclohexyl-barbituric acid	250	4.6	1.8
none	—	1.7	5.0
1-cyclohexyl-5,5-diallyl-barbituric acid	250	4.2	2.0
none	—	2.0	4.8
3-methyl-5-benzoyl-aminoiso-thiazole-4-carboxy-p-chloro-phenylamine	12	3.7	2.5
none	—	1.7	6.1
3-methyl-5-benzoyl-amino-isothiazole-4-carboxy-p-ethoxy-phenylamine	12	5.2	1.7
none	—	1.5	5.5

In all experiments the chick embryo tissue cultures were incubated for 2 days at 37 °C. The doses of the drugs used were those giving the maximal effect. n.d. = not done.

large doses of salicylate produce a profound decrease in glucose concentration and accumulation of lactic acid in the brains of young mice. Whereas in the blood the deficiency of glucose is immediately compensated by glycogenolysis.

Recently also DOERY and HIRSH[5] investigated the effect of the therapeutic levels of aspirin and salicylate on the glycolysis of platelets. They suggested that the drugs have divergent action: aspirin caused a fall in glucose concentration in the medium whereas salicylate was without effect. We believe that the lack of activity of salicylate in their experiments may be due to the application of too low concentration of the drug since salicylate is almost twice less active than aspirin e.g. in the test of stabilization of human erythrocytes[11].

We suggest that the determination of lactic acid production and glucose uptake in tissue culture may be useful for the preliminary screening of drugs for the anti-inflammatory activity.

Zusammenfassung. In der Kultur embryonalen Hühnchengewebes kommt es unter Einfluss nichtsteroider Antiphlogistica (Aspirin, Indomethacin, Mefenamicsäure) zu einer Steigerung des Glukoseverbrauchs bei gleichzeitiger Vermehrung der Milchsäureerzeugung. Der Milchsäureverbrauch verläuft in zwei Phasen, wobei der erste Gipfel bei geringsten Konzentrationen des Arzneimittels auftritt.

ELZBIETA WOLNA and ANNA D. INGLOT

Department of Biochemistry, Institute of Biochemistry and Biophysics, Medical School, Wroclaw; and Department of Virology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Chalubinskiego 4, Wroclaw (Poland),
17 May 1972.

[11] A. D. INGLOT and E. WOLNA, Biochem. Pharmac. *17*, 269 (1968).

Effects of Nicotine and Arousal on the Monkey Electroencephalogram

The effects of intravenous administration of intermittent doses of nicotine and oral doses of caffeine and D-amphetamine have been investigated on the EEG of the conscious squirrel monkey. The intermittent i.v. injection for nicotine was considered to closely simulate the uptake of nicotine during smoking, whilst the oral route for caffeine and amphetamine represented the normal route of administration for these drugs.

The results reported suggest that smoking doses of nicotine may produce an EEG state close to that obtained in normal alert situations; this is in contrast to that induced by the other centrally acting stimulants D-amphetamine and caffeine, suggesting an abnormal state after administration of these drugs.

Method. Twelve adult squirrel monkeys of either sex were used in this study. Platinum electrodes were implant-

Table I. Differences from control values in total EEG activity of the squirrel monkey EEG (left frontal-left occipital) following different drug treatments

Treatment	No. of animals	No. of experiments	Total EEG activity % change from control	Significant difference from arousal state (p)
Arousal	11	35	−41.1	
Saline	7	9	− 4.3	< 0.01
Nicotine (1 μg/kg/min i.v.)	10	11	−11.9	< 0.01
Nicotine (5 μg/kg/min i.v.)	6	8	−37.1	
D-amphetamine (0.1 mg/kg p.o.)	5	5	−17.6	< 0.01
D-amphetamine (0.5 mg/kg p.o.)	5	5	−50.8	
Caffeine (50 mg/kg p.o.)	5	8	−34.7	

Total EEG activity for nicotine was determined in the last 9 min of a 20 min intermittent i.v. injection period, and for caffeine and D-amphetamine during the 26–35 min following oral dosing.