Arterial blood pH and gas tensions in guinea-pigs[a]

	Unanesthetized Animals (Group I)[b]				
	Present Data	Data of HAWKINS[c]	Group II[b]	Group III-A[b]	Group III-B
pH	7.50 ± 0.02	7.35 ± 0.03	7.40 ± 0.03[e]	7.50 ± 0.02	7.49 ± 0.03
pCO_2	31 ± 1	33 ± 3[d]	47 ± 3[e]	32 ± 2	—
pO_2	81 ± 2	—	73 ± 5	85 ± 3	—
n[f]	15	12	11	10	10

[a] Values represent mean ± S.E. [b] Designation of groups: Group I: unanesthetized, spontaneously breathing; Group II: anesthetized, spontaneously breathing; Group III-A: anesthezied and artificially respired at normal pressure; Group III-B: anesthetized and artificially respired at 282 ψ. [c] Blood obtained by cardiac puncture[1]. [d] Reported as volume percent CO_2 and converted to pCO_2 by standard formula[4]. [e] Significantly different than values for animals in Group I ($p < 0.01$, Student t-test). [f] n = number of animals.

the 1-hour sample, served as a guide for adjusting the rate of artificial respiration for the remainder of the experiment.

Blood pH, pCO_2, and pO_2 were measured with an Instrumentation Laboratories Model 113 analyzer. In animals of group III-B, only pH could be measured accurately because effervescence in decompressed blood interfered with measurement of pCO_2 and pO_2. A 2-ml sample of blood was sufficient for the analyses. Results for the 4 groups of animals are shown in the Table. The data demonstrate that anesthesia significantly depressed respiration in spontaneously breathing guinea-pigs (group II) as compared with normal animals (group I). However, the Table also shows that the artificial respiration used in these studies (groups III-A or III-B) successfully reversed the respiratory depression and maintained the animals in normal acid-base balance. Apparently, artificial ventilation was not appreciably affected by the use of a gas mixture that was approximately 2.9 times as dense as air at a pressure of 1 atm.

It is interesting to compare the present data with previously published values for pH and pCO_2 in guinea-pigs, also given in the Table. Currently cited values for normal guinea-pigs[1] are based on the work of HAWKINS[3], who analyzed blood obtained by cardiac puncture, using the Van Slyke method to determine total CO_2, and a comparator block method to estimate pH. Results obtained with cardiac puncture blood should be interpreted with caution because of the likelihood of aspirating a mixture of arterial and venous blood. The Table shows that HAWKINS reported nearly the same pCO_2, but a much lower pH than was found in the present study.

In conclusion, the present data demonstrate that artificial respiration of guinea-pigs, as performed in this labofatory, provided adequate ventilation of the animals, both at normal pressure and in a hyperbaric helium atmosphere. In addition, the pH and pCO_2 reported here for unanesthetized animals may be closer to true values for normal guinea-pigs than are the currently cited values[5,6].

Zusammenfassung. Säure-Basen-Werte von Meerschweinchen (*Cavia porcellus* L.) wurden unter künstlicher Atmung bei normalem Druck und bei Druck von 19.2 Atm. gemessen und festgestellt, dass künstliche Atmung unter erhöhtem Druck normale Säure-Basen-Gleichgewichte gewährleistet, während die Säure-Basen-Werte normaler Tiere mit denjenigen der Literatur nicht übereinstimmten.

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[6] Experiments reported herein were conducted according to the principles enumerated in *Guide for Laboratory Animal Facilities and Care*, prepared by the committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council, Washington, D.C.

Dopa and Dopamine in the Pigment of Substantia Nigra

The cells of substantia nigra contain a pigment which is generally thought to be a melanin. Investigations using histochemical methods, electron microscopy and IR-spectroscopy have, however, revealed several differences between the substantia nigra pigment and other melanins[1-5].

The chief structural difference between substantia nigra and extra-neural melanin granules is the presence of lipid globules in the melanin granules of Substantia nigra. The chemical nature of Substantia nigra melanin is unknown, but it has been suggested that the pigment is formed from dopa, dopamine, adrenaline, noradrenaline or 5-hydroxytryptamine[6-9].

Although the structure of different melanins is very complex, it is possible to get an idea of the nature of different melanins by degradation experiments[9]. Therefore Substantia nigra melanin was hydrolyzed in hydrochloric acid and the hydrolysate investigated for dopa, noradrenaline, adrenaline, dopamine and 5-hydroxytryptamine.

Material and methods. Substantia nigra was obtained at autopsy from 16 humans. The tissue specimens were homogenized in 6 N HCl (5 g tissue in 20 ml acid) and

centrifuged for 10 min at 17,000 *g*. The sediment was resuspended and centrifuged in 6 *N* HCl 3 times and thereafter hydrolyzed in 6 *N* HCl for 7 h. The last supernatant and the hydrolysate were examined for the following substances: A) Dopa, according to ANTON and SAYRE[10]. B) Adrenaline and noradrenaline, according to BERTLER et al.[11]. C) Dopamine, according to CARLSSON and WALDECK[12]. D) 5-hydroxytryptamine, according to BERTLER and ROSENGREN[13].

Results and comments. The determination of dopa and the various biogenic amines showed that dopa and dopamine could be recovered in the hydrolysate of Substantia nigra melanin. When hydrolysis was repeated 3 times, only dopa was found. No dopa, dopamine or other amines were present in the last supernatant after repeated washing of melanin in 6 *N* HCl.

Dopamine has not previously been found in naturally occurring melanin, but under experimental conditions dopamine may form melanin, and knowledge of this melanin is to a considerable extent thanks to the work of SWAN[14]. According to the classic Raper-Mason concept, melanin formed from dopa or dopamine should contain only indole-quinones, but it is now thought that dopa or dopamine can be incorporated in the melanin[14–17].

The presence of dopamine in Substantia nigra melanin is of great interest, since dopamine has been demonstrated in considerable amounts in this structure[18]. Dopamine is believed to have a special function in the extra-pyramidal system, and the dopamine-containing nerve terminals of the putamen and of the caudate nucleus probably originate in Substantia nigra[19]. It is noteworthy that pigment forms only in this structure, while the dopamine concentration is 10-fold higher in the caudate nucleus and the putamen[18, 20].

Various explanations may be offered for the presence of dopa and dopamine in Substantia nigra pigment. Pigment granules may be formed by oxidation of tyrosin and dopa and polymerization of the oxidation products. Then dopa may be copolymerized with the polymerized indole-quinones. Dopamine available in Substantia nigra cells might then also be copolymerized. It is also possible that pigment granules are formed by oxidation of dopamine and polymerization of the formed indole-quinones. Dopamine, but also some still undecarboxylated dopa, may then be copolymerized.

Finally, it cannot be excluded that the Substantia nigra melanin is a mixture of dopa melanin and dopamine melanin in which case dopa and dopamine should be present in different granules.

The finding of dopa only, after 3 hydrolyses of 7 h each, favours the view that the primary nucleus of melanin formed is dopa melanin, while the dopamine melanin forms a shell on the melanin particles[21].

Zusammenfassung. Hydrolysate der Pigmentpartikel der Substantia nigra enthalten Dopa und Dopamin, jedoch kein Adrenalin, Noradrenalin oder Serotonin.

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Inhibitory Effect of Proteolytic Enzymes on Platelet Aggregation Induced by ADP or Thrombin

Although a number of substances have been reported to inhibit platelet aggregation or adhesion, most of them should be inquired more to be approved as reliable and available in clinical use on thromboembolism. One of these categories includes proteolytic enzymes. The present study describes alterations in the sensitivity of unwashed and washed platelets with ADP and thrombin when platelets were treated with proteolytic enzymes.

Materials and methods. Human venous blood anticoagulated with 10% by volume of 8% trisodium citrate was centrifuged at 170*g* for 30 min at room temperature to obtain platelet-rich plasma (PRP). The platelet concentration in PRP was adjusted by using platelet poor plasma (PPP) as a diluent to $25 \times 10^4/mm^3$ employing BRECHER-CRONKITE method[1]. All glasswares were siliconized. 50 mg of each proteolytic enzyme: protease (1,900,000 U/g), by courtesy of Pacific Lab., Inc., Honolulu, HI.; papain (1000 GDU (gelatin digesting unit)/g, and bromelain (1200 GDU/g), provided by Dr. S. TAUSIK) Director of Research, Dole Co., Honolulu, HI.; ficin (200–800 U/g), obtained from Sigma Chemical Co. (St. Louis, Mo.), was dissolved in phosphate buffered saline (PBS) pH 7.2 to yield the concentration of 20 and 200 µg/ml. PRP was incubated with an equal volume of each enzyme solution in a plastic tube at 37 °C for 30 min. The mixture of PRP and PBS was used as a control.

The ADP sensitivity test of platelets was performed by method of YAMAKIDO et al.[2] as modified by SANO et al.[3, 4]. The principle of the method is to obtain the