amount of ACTH have previously been observed[12,13]. BANERJEE and GHOSH[14] have reported the histochemical changes in testes and adrenal glands during scurvy to be corrected on administration of ascorbic acid. DEB and BISWAS[15] have suggested that the inhibition of spermatogenesis in rats produced by high tyrosine diet was possibly due to a reduced secretion of hypophyseal gonadotropin, although they have noted a correction of the said disturbances on vitamin C. The functional activity of the pituitary gland in ascorbic acid deficiency has not been studied in detail. DEB and BANERJEE[16] have noted a fall in alkaline phosphatase in the anterior pituitary during scurvy and postulated it to be possibly due to diminution of some pituitary trophic hormone. Recently, pituitary bioassay revealed that the gonadotropic potency has been increased in C-deficient guinea-pigs (DEB and BISWAS[17]). An increased gonadotropic content and reproductive disturbances also have been reported in B_6-deficient rats[18,19]. The testicular degeneration in ascorbic acid deficiency may, therefore, be caused by failure of the pituitary to release sufficient gonadotropic hormone[20].

Résumé. La dégénération des testicules de cobayes manquant d'acide ascorbique a pu être corrigée par l'administration du sérum gonadotropin.

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Effect of Silica on Protein Biosynthesis by Rat Liver Cell-Free Systems

Silica particles engulfed by macrophages in vitro bring about an injurious effect to these cells. The mechanisms of cytotoxic action of silica and their specificity have been widely investigated in recent years on cultured macrophages and on various cells and tissues by several workers[1–8]. Nevertheless, the nature of the toxic effect of silica remains a problem still open to question.

The present report is a further study[9,10] on the same question. It deals with the action of particulate silica on protein biosynthesis at subcellular levels by investigating the amino acid incorporation rate into protein by rat liver cell-free extracts.

The microsomal system of ZAMECNIK and KELLER[11] as described by STEIN and GROSS[12] has been used, and purified preparations of crystalline HF-treated silica (tridymite) and of vitreous HF-treated silica (both in a diameter range $\sim 1\ \mu$) tested. Liver homogenates 1:3 by volume were prepared with teflon pestle homogenizer in $0.25\,M$ sucrose with $0.025\,M$ $KHCO_3$, $0.02\,M$ potassium phosphate buffer pH 7.8 and $0.01\,M$ nicotinamide. Nuclei and cell debris were removed by centrifugation at 800 *g* for 6 min and the resulting supernate recentrifuged for 15 min at 10,000 *g*. Centrifugations were made in a refrigerated International centrifuge. 1.0 ml of the 10,000 *g* supernate was incubated in Warburg flasks with 20 μM fructose 1-6 diphosphate potassium salt, 2.0 μM ATP potassium salt, 2.0 μM $MgCl_2$ and 0.26 μM DL-leucine-1-C^{14} (specific activity 3.8 $\mu c/\mu M$) in a final volume of 2.0 ml. When present silica was in amount of 15 mg. Incubations were carried out for 1 h at 37°C in a Warburg metabolic shaking incubator with O_2 95% + CO_2 5% as gas phase. The reaction was stopped by TCA and protein

In vitro incorporation of DL-leucine-1-C^{14} into rat liver microsomal protein. Each figure (c.p.m. per mg protein) is the mean ± S.E. of 2 flasks

Experiment	Control	Crystalline silica	Vitreous silica
1	92.5 ± 4.47	52.0 ± 1.25	–
2	80.5 ± 5.48	59.0 ± 5.00	–
3	116.0 ± 3.00	62.0 ± 1.40	–
4	111.0 ± 9.00	65.0 ± 6.00	–
1	81.0 ± 3.00	–	65.5 ± 7.48
2	75.5 ± 10.49	–	43.0 ± 6.00
3	84.0 ± 10.00	–	76.5 ± 3.46
4	80.5 ± 5.48	–	62.5 ± 7.48

The statistical evaluation of data has been made with the analysis of variance test.

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isolated using the procedure of SIEKEVITZ[13]. Calculated portions of dried protein were plated on stainless steel planchets and counted dry with a thin mica end window G.M. counter. Counting error was less than 2%. Protein was estimated after nitrogen determination with Nessler reagent.

From the data given in the Table it can be seen that leucine incorporation into the microsomal protein by rat liver homogenates is depressed by tridymite ($P < 0.001$): conversely, vitreous silica seems to carry out a less definite action in that sense ($P < 0.05$). It is to be noted that there is a large fluctuation in the experimental data. This is the result of the combined biological and analytical errors, a phenomenon which might also obscure the experimental response if data were incorrectly analysed.

The observations here reported are in keeping with those of ZAMECNIK and KELLER[11] as far as the incorporation rate in control cell-free systems is concerned. The observations show the presence of an inhibitory action by silica dusts on protein biosynthesis at subcellular levels. This action is more pronounced in the presence of crystalline than vitreous silica, an effect which might possibly be related with the well-known less toxic, in vitro, and fibrogenic, in vivo, properties of the latter dust. These observations may actually confirm the derangement of protein biosynthesis[9], a possible specific action of silica in tissues.

Particulate silica in the form of quartz is known to adsorb protein material (e.g. serum proteins and enzymes) and amino acids on its surface[14,15] and to have also oxidative and hydroxylative properties on these latter compounds[16,17]. The decreased protein radioactivity found by the experiments here reported might be the consequence of the peculiar surface activity of particulate silica. As a matter of fact, the extent of the observed inhibition of the amino acid incorporation into the microsomal protein seems to be related to the surface structure of the silica particle tested[18].

Riassunto. È stata studiata l'azione di particelle di silice a struttura cristallina e non cristallina sulla incorporazione di leucina-1-C^{14} nelle proteine microsomiali di fegato.

La silice inibisce l'incorporazione, ma con diversa intensità. Ciò appare essere in rapporto con la struttura delle particelle impiegate.

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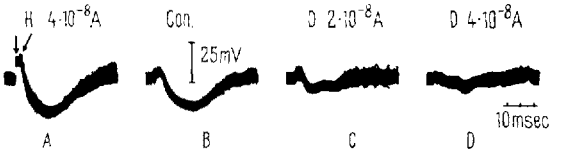
'Disfacilitation' of Red Nucleus Neurones

It has been reported[1] that the large neurones of the red nucleus (RN) receive powerful excitatory postsynaptic potentials (EPSPs) monosynaptically from the contralateral nucleus interpositus (IP) of the cerebellum. On the other hand, stimulation of the cerebellar cortex provokes inhibitory postsynaptic potentials (IPSPs) monosynaptically in the IP neurones, presumably via the corticofugal axons of cerebellar Purkinje cells[2]. Thus it may be expected that the cerebellar cortex acts upon the RN indirectly by inhibiting the IP. This process of disfacilitation, as it may be called, has indeed been demonstrated in the present work.

Cats were used under light nembutal anaesthesia. The experimental procedures were essentially the same as those employed previously[1]. The RN cells were impaled with glass-microclectrodes containing $2M$ NaCl and having electrical resistance of 10–20 $M\Omega$.

In the case of the Figure A–D, the IP region was stimulated with needle electrodes. EPSPs were first induced at latencies of 1.0 and 2.2 msec, respectively (indicated by vertical and oblique arrows), presumably by direct and transsynaptic activation of IP neurones. Thereafter, starting at 6.7 msec a large hyperpolarization occurred, lasting over several ten-milliseconds (Figure B). These observations are in agreement with those by MASSION and ALBE-FESSARD[3,4]. When the membrane potential was displaced by applying hyperpolarizing (Figure A) or depolarizing (Figure C, D) currents, the amplitude of this membrane hyperpolarization was increased or decreased (control in Figure B) in parallel with that of EPSPs. In Figure D the hyperpolarization virtually disappeared together with the EPSPs, indicating that the nul-potential of the hyperpolarization is the same as that of EPSPs. Consequently, the membrane hyperpolarization should not be the IPSP but it should be due to removal of tonically induced EPSPs in RN neurones. Membrane hyperpolarization of the same nature has been seen in ex-



Membrane potential changes produced in a RN neurone by stimulation of IP region. A, during application of hyperpolarizing currents of $4 \cdot 10^{-8}$ A; B, control; C and D, during passage of depolarizing currents of $2 \cdot 10^{-8}$ A and $4 \cdot 10^{-8}$ A, respectively. All records were taken by superposing about 20 faint traces.

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