not found in leptotene, zygotene, pachytene and diffuse stage. Diplotene and diakinesis are passed quickly. Thereafter the meiotic divisions are highly abnormal like in *C. morosus*[3,8].

Oogenesis in the end chambers of the ovarioles of the ovotestes proceeds with the same morphological stages observed in the end chambers of ovaries. Thus, heterochromosomes are not present, a slight heteropycnosis of some pachytene bivalents is evident, and an additional duplication of chromosomes takes place after pachytene.

These results show that in oocytes the chromosomes are duplicated immediately after pachytene, like in *C. morosus*, and thus give the 2nd example of this peculiar doubling system. The additional duplication makes possible the parthenogenetic reproduction. In the ovotestes, the female germ cells are morphologically similar to those in ovarioles of ovaries, and the male germ cells show the same phenotypic features as those of other stick insect species[3]. This situation is consistent with the current opinion that the phenotypic sex of the germ line in insects is determined by its chromosome constitution[9,10], admitting that our exceptional male obtained through irradiation probably was mosaic in origin.

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Glutathione peroxidase in dried blood spots

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Summary. A new procedure utilizing dried blood spots was developed for detecting glutathione peroxidase deficiency. Samples from a known patient with a partial defect and from rats with an induced deficiency were distinguished from respective control groups by their longer defluorescence endpoints. Samples from 100 patients with anemia and 2 phenylketonuric infants on low-protein diets contained glutathione peroxidase activity similar to that in 82 controls, when screened for the enzyme defect by the new procedure.

We describe a screening method for glutathione peroxidase, an enzyme, which, in vitro, catalyzes the detoxification of peroxides via glutathione in the hexose monophosphate pathway. The techniques were adapted from Beutler's fluorescent tests for other red cell enzymes[1], modified to the kinetics of peroxidase[2] and applied to the dried blood spot sample. The test should facilitate the identification of patients with peroxidase defects, only a few of whom have been described[3-6]. In our test, peroxidase activity (GSH-Px) is indicated by defluorescence, as reduced nicotinamide adenine dinucleotide phosphate (NADPH) is oxidized to NADP in a linked reaction with glutathione (2GSH < − > GSSG).

$$2\,GSH + H_2O_2 \xrightarrow{GSH\text{-}Px} H_2O + GSSG \xrightarrow{GSSG\text{-}R} 2\,GSH$$

$$NADPH \rightarrow NADP$$

Material and methods. Defluorescence times of dried blood spot samples from I.S., a patient with a partial deficiency of glutathione peroxidase[5] (personal communication from Professor Rodolfo Bracci) were compared with those of mailed spots from 40 newborns being screened for inborn errors in a state-wide program and from 100 patients with unexplained anemia, varying in age from infants to adults, and with those of fresh spots from 11 healthy laboratory workers, aged 23–55, and 34 adults undergoing blood tests as outpatients for a variety of illnesses.

The samples were obtained by saturating filter paper cards (3MM) with spots of blood and air-drying for at least 1 h. In the procedure, discs of 5 mm diameter were punched from the cards, guided into the wells of a plastic tray and overlaid with 0.2 ml reaction mixture. The solutions were mixed well with plugged hematocrit tubes. The reaction mixture contained the following ingredients prepared ahead of time: 1 ml 1 M potassium phosphate buffer, pH 7.0, 0.2 ml 0.2 M ethylenediaminetetraacetic acid, 0.1 ml 0.4 M sodium azide, 0.2 ml ferricyanide-cyanide solution (30 mg $K_3Fe(CN)_6$ + 10 mg NaCN/100 ml water) and 1.4 ml demineralized water were combined and stored for several days at −20 °C before use. The following ingredients, however, were prepared fresh and added to the solution above just before use: 0.1 ml 0.1 M glutathione (GSH), 1.0 ml glutathione reductase (10 U/ml Sigma), and 1.0 ml 2 mM nicotinamide adenine dinucleotide phosphate, reduced (NADPH). 25 λ H_2O_2 (5 mM) were added to the reaction mixtures in the wells and mixed. Aliquots of the incubation mixture were applied as single drops to a premarked Whatman No. 1 filter paper sheet immediately, mixed with a plugged microhematocrit tube and air-dried for at least 30 min. Incubation mixtures were incubated at 37 °C for at least 10 min and aliquots removed, spotted at 3-min intervals and air dried. Defluorescence was observed in a long-wave UV view box. Incubation mixture without a blood spot sample served as the positive (enzyme-deficient) control.

The accuracy of the method was ascertained by comparing defluorescence times in the blood spot samples with enzyme activities in the whole blood samples from 13 of the adult donors undergoing blood tests from whom the spot

Defluorescence times and activities of glutathione peroxidase in 13 whole blood and spot samples from adult outpatients

Defluorescence time (min)	Peroxidase activities (IU/g Hb)
3	6, 7, 7, 8, 11, 7
6	3, 4, 5, 5, 6, 6, 6, 5
9	3, 3

samples were made as measured by Beutler's quantitative method[7].

The specificity of the reaction was ascertained by omitting single reagents and comparing defluorescence times with those of samples incubated with the complete mixture.

The stability of the reagents in storage was verified by comparing the defluorescence times of reactions using freshly-prepared mixtures with those using mixtures stored at −20 °C for up to 4 weeks. The stability of enzyme was tested by comparing endpoints of dried blood spot samples left at room temperature (21–25 °C) for up to 1 month with those of samples stored at −20 °C.

The stability of enzyme in dried blood spot samples of heparinized venous blood was compared with that in comparable samples of capillary blood without anticoagulant. Both groups of samples were stored at room temperature and at −20 °C, as above.

The possibility that the new test might be useful in detecting selenium deficiencies in infants receiving synthetic diets[8] was explored by comparing defluorescence times and enzyme activities in spot and whole blood samples from phenylketonuric infants who had been receiving synthetic diets for several months.

The new test was adapted to rat blood, with the intent that it be a convenient means of detecting selenium deficiencies in animals fed restricted diets. Rat blood peroxidase is inversely related to dietary selenium levels, and selenium has been closely associated with the structure of rat and sheep peroxidase[8]. The test conditions studied were concentration of NADPH and length of incubation period.

Activity in blood spots from 68 control rats was compared with that from 3 rats made glutathione peroxidase-deficient by a selenium-deficient diet, kindly sent by Roger A. Sunde of the University of Wisconsin[8]. The control rats consisted of 47 healthy, untreated rats and 21 rats, treated in the course of other experiments, 8 of which had received phenobarbital injections, 6 oral warfarin and 7 oral coconut oil.

Results. Reaction mixture containing peroxidase from the dried blood spot samples defluoresced with increasing incubation time (figure 1). Four-fifths of the fresh and newborns' (mailed) samples defluoresced completely (black spots) after 3 min incubation. Several of the mailed samples (newborns') and 8 of the fresh spots required 6 min; 1 mailed and 2 fresh samples required 9 min incubation. Samples from the 2 treated phenylketonuric infants required 6 and 9 min, respectively. The sample from the partially peroxidase-deficient patient defluoresced in from 10 to 12 min (figure 1). Mixtures from which blood spot samples were omitted and therefore contained no enzyme (positive control) remained fluorescent (bright, blue-green spots) throughout incubation periods of up to 40 min.

The endpoints were roughly consistent with peroxidase activities in the corresponding whole blood samples. The shortest defluorescence times, for example, coincided with the greatest activities, and the longer endpoints coincided with lesser activities (table). The longer endpoint for the sample from the partially peroxidase-deficient patient was consistent with the partial activity in her whole blood (7.2 IU/g Hb, compared to normal activity of 11.6±1.1 IU/g Hb., personal communication from Dr Rodolfo Bracci). Glutathione, glutathione reductase and hydrogen peroxidase were considered essential to the reaction, as mixtures from which they were omitted individually remained fluorescent, despite incubation periods of up to 40 min.

We also found that several reagents could be prepared

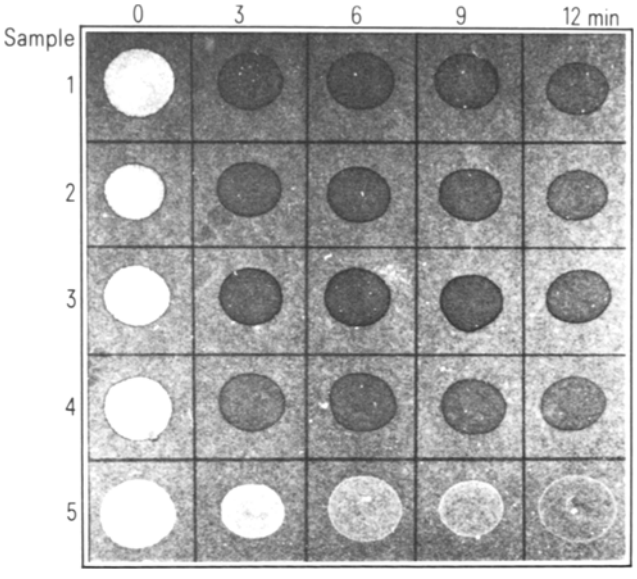

Fig. 1. Defluorescence time of glutathione peroxidase in dried blood spots from healthy adults (sample 1–4) and patient with partial deficiency of the enzyme (sample 5).

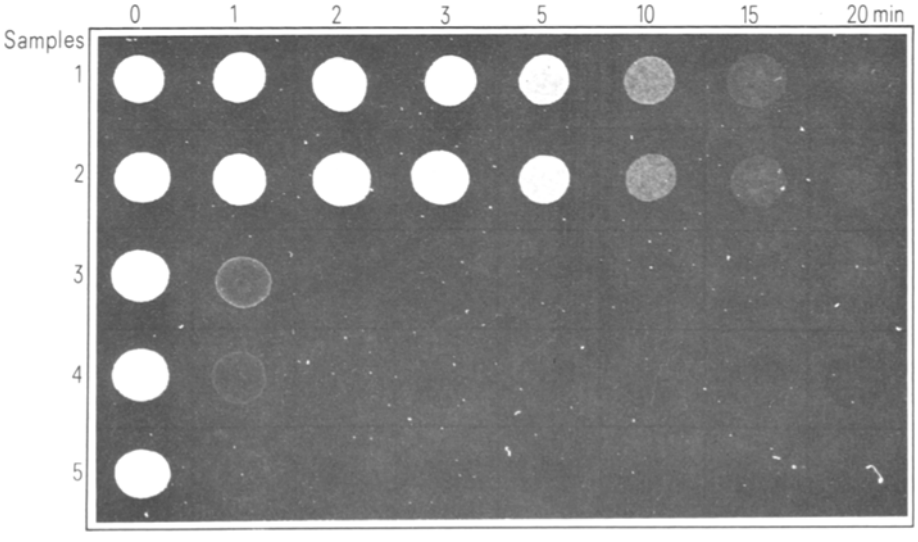

Fig. 2. Defluorescence time of glutathione peroxidase in dried blood spots from rats with selenium deficiencies (samples 1 and 2) and from mailed control (sample 3) or stored control (samples 4 and 5).

ahead of time and stored at −20 °C for as long as 4 weeks, without prolonging defluorescence endpoints beyond those obtained with freshly-prepared mixtures. Longer storage was detrimental, however, as endpoints with older mixtures were delayed by several minutes.

Peroxidase was relatively stable in the dried blood spot sample, as defluorescence endpoints in samples stored at room temperature for up to 1 month were similar to those in samples stored at −20 °C for the same period. Samples stored at −20 °C, furthermore, retained their activity for up to 3 months. Endpoints in dried blood spot samples made from heparinized venous blood were similar to those in samples of capillary blood without anticoagulant when stored as above. Defluorescence endpoints and enzyme activities in the samples from 4 phenylketonuric infants receiving dietary treatment were similar to those of the other samples tested, i.e., endpoints of 6 and 9 min and activities of from 3.1 to 8.2 IU/g hemoglobin. Samples from rats appeared to contain far more enzyme than human blood in tests with the same substrate, in which defluorescence endpoints of the rat blood samples were reached in less than 1 min. The test was modified, therefore, to make it more discriminatory for rat blood peroxidase, by delaying the appearance of endpoints. By doubling the concentration of TPNH in the substrate to 4 mM, the time required for completion of the reaction, i.e., oxidation (defluorescence), was lengthened. Using the more concentrated substrate, activities in dried blood spots from control rats were compared with those in spots from selenium-deficient rats. Defluorescence endpoints in the control groups, in all instances, were 3 min or less, in contrast to those from the deficient rats, which required 20 min. Corresponding whole blood peroxidase activities in healthy, untreated control rats and in selenium-deficient rats were 0.428 and 0.090 IU/mg Hb, respectively (personal communication from Roger A. Sunde) (figure 2).

Discussion. The methodology described adds yet another enzyme to the list of those detectable in dried blood spots. The advantages of the new test are the peroxidase's stability in the sample, the small volume of blood required, the convenience of mailing the sample and the assay's rapidity and simplicity. These features should facilitate screening for the defect, the data of which may be used to identify the homozygous phenotype, estimate the defect's frequency and distribution and assess its role in the etiology of hemolytic anemia. Investigators should be aware, however, that partial peroxidase deficiencies are sometimes found in normal infants[3] and should consider the possibility, at the other extreme, that peroxidase deficiencies may be induced following treatment with purified diets deficient in selenium[8].

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Pollution selection of allozyme polymorphisms in barnacles[1]

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Summary. Allozymic variation in proteins encoded by 15 loci was analyzed electrophoretically in 166 individuals of the subtropical acorn barnacle *Balanus amphitrite* from 3 sites varying in pollution levels, situated within 3 km of one another in the Mediterranean Haifa Bay. The 3 sites respectively were a relatively unpolluted marine bay, a petroleum polluted port, and a petrochemically polluted dockyard. Out of the 15 loci tested, 10 exhibited in both 1974 and 1975 statistically significant repetitive trends in allele frequencies in accord with the 3 sites. It is hypothesized that natural selection presumably favours specific alleles in each site, and that in barnacles different allozymic variants function optimally in different polluted environment.

Marine pollution and its effects on living resources[3], including the possible involvement of enzyme polymorphisms such as oxidases and hydrolases, in detoxification of pollutants and sewage has been extensively studied[4–6]. Yet the more general problem of how and to what extent genetic variation found in natural populations is utilized as a basis for adaptive evolution in changing environments is still unanswered[7]. Direct correlation of isozymes with the environment is one promising approach to elucidate this substantive yet controversial problem of evolutionary genetics. We tested the effect of pollution on barnacles in an attempt to assess the role of allozyme polymorphisms in fitness. Our results suggest that allozymic variation is at least partly adaptive in the subtropical acorn barnacle *Balanus amphitrite.*

Materials and methods. Acorn barnacles, genus *Balanus,* are nearly cosmopolitan marine crustaceans. Adult barnacles, following larval settlement, are stationary and directly exposed to local environmental variation. We sampled 3 sites varying in pollution levels situated within 3 km of one another in the Mediterranean Haifa Bay (figure 1). Quiet Beach is a regular marine bay, and represents the least polluted site. The Oil Terminal is petroleum-polluted due to discharge from tankers. The Kishon Dockyard, near the Kishon River outlet, is the most polluted site, containing industrial, sewage, and petroleum wastes discharged from dozens of plants, oil refineries, petrochemical and chemical industries. The Kishon site included, according to a 1975 unpublished survey[8], 2 categories of pollutants, a) mineral, amounting to a daily discharge of 6700 m^3, highly acidic