efficiency." "This level—90 dB—is not at all unusual in industry, but it is very much higher than the level which people often complain about in offices and other places away from the factory floor." As is common experience, undue noise makes normal conversation impossible; it is in fact difficult unless the noise is reduced well below the 90-dB level. 60 dB is considered a more reasonable level, but in an office environment, where ordinary speech is involved, the limits of noise should be much lower.

The question of noise in all its aspects, but particularly as a hazard in industry, is now a matter of national importance. It is indeed gratifying to learn that the Ministry of Pensions and National Insurance has sponsored a large-scale research project into certain aspects of occupational deafness. This is the joint problem of the Medical Research Council and the National Physical Laboratory. "The purpose of this study is to monitor hearing of people exposed to industrial noise . . . to establish the relation between noise and damage to hearing . . . to make recommendations concerning hearing-conversation measures." When it is commonly realized that effects of prolonged noise, in whatever environment, above a certain level, are real, and provocative of permanent deafness, then people may begin to take those precautions which common sense (and this booklet) suggest. Apart from this, undue and unwanted noise, especially when it intrudes into social life, public or private, without let or hindrance, is a form of annoyance which many people will not tolerate. The recent ban on transistor radio sets operating in some of Britain's royal parks is a step in the right direction, but only a beginning.

H. B. MILNER

THE LISTER INSTITUTE

THE annual report of the Governing Body of the Lister Institute* is necessarily a greatly condensed account of the varied activities of 'The Lister', and it is impossible to indicate in a brief notice the scope and detail of the research involved. The report summarizes the Institute's work under several headings.

In the field of microbiology, work continues on the genetics of bacteria, particularly *Salmonella* spp., with special regard to the transmission of drug resistance; on the specificity of the antigens of *Trypanosoma brucei* and *T. vivax*, and on the conditions governing the formation of capsules by the anthrax bacillus *in vitro*. The Institute's field studies on trachoma in the Gambia continue, together with work on the epidemiology and pathology of pleuropneumonia-like organisms and of the Shope fibroma virus in cell culture. There has been continued progress in making a non-infectious smallpox vaccine and in work on the purification of vaccinia virus and antibody immunity in vaccinia and variola infections.

Among the epidemiological studies was an important investigation, carried out in co-operation with the Malaria Eradication Division of the World Health Organization and the Veterinary Services of Southern Rhodesia, of identification tests of the blood meals of tsetse flies and *Anopheles* mosquitoes.

Immunopathological investigations are being carried out on a factor in anaphylactic tissues which induces eosinophilia; on the local production in the cervix uteri of antibodies lethal to spermatozoa, and on improvement of therapeutic antitoxins to scorpion bites.

T. brucei, which had not been previously cultured for any prolonged period, has been successfully established in a liquid medium which makes it available for immunological and biochemical studies. Other work includes investigations of the immunology of whooping cough and typhoid and the mechanism of infection with actinomycotic dermatitis of sheep. The section devoted to the physiology of bacteria includes studies of the somatic polysaccharides of S. and R. *Salmonella* and the chemistry of the flagellar proteins of *Salmonella*.

Biophysical work includes work on human plasma proteins and on the molecular characterizations of blood-group substances. The latter has led to extension and modification of existing theories for interpreting sedimentation and viscosity data. The Blood Products Laboratory continued to prepare dried plasma and plasma fractions for the National Health Service, and the Blood Group Research Unit has continued to study the Xg and the Rh systems. The Blood Group Reference Laboratory continues its work in its new building north of the Biophysics Department.

The report records the conferment of a baronetcy on the chairman of the Institute's Governing Body, Sir Charles Dodds, and the elections of Lord Iveagh and Lord Brain, both members of the Governing Body, to fellowship of the Royal Society. The death during the year of R. St. John Brooks, curator for more than 20 years of the National Collection of Type Cultures, is a great loss to the Institute and to microbiology in general. The director of the Institute, Prof. A. A. Miles, has been appointed Biological Secretary and a vice-president of the Royal Society of London. During the year the Guinness chair of microbiology was established at the Institute and Dr. B. A. D. Stocker was appointed to it.

A gift of £30,000 from the Fleming Memorial Fund for Medical Research will enable the Institute to establish its own electron-microscope unit. The new Virus Laboratory at Elstree was completed during the year and occupied by the Virus Research Unit, and the Library at Chelsea was enlarged by the addition of another room.

The report also records the lectures given by the staff in Europe and America, and concludes with a list of their numerous publications.

For details of the biochemical work on blood-group substances, cellular phospholipids and glycogen the report itself must be consulted.

G. LAPAGE

* *The Lister Institute of Preventive Medicine.* Report of the Governing Body, 1964. Pp. 37. (London: The Lister Institute of Preventive Medicine, 1964.)

MOUSE ANTIBODY PRODUCTION TEST FOR THE ASSAY OF THE MOLONEY VIRUS

By DR. EVA KLEIN and PROF. GEORGE KLEIN

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INOCULATION of homografts, sub-threshold isografts or tissue homogenates derived from mouse lymphomas induced by the Moloney virus leads to specific transplantation resistance against subsequent Moloney lymphoma isografts[1]. The serum of resistant mice contains humoral antibodies, demonstrable in three different ways: by passive transfer of transplantation resistance, by cytotoxicity for Moloney lymphoma cells *in vitro* in the

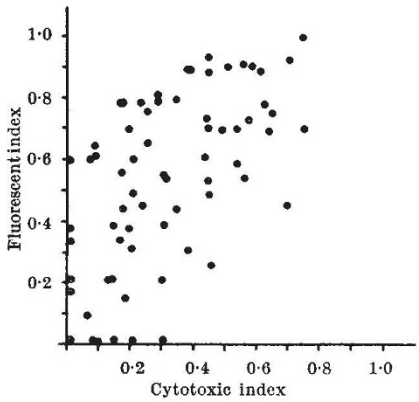

Fig. 1. Correlation between the cytotoxic and the fluorescent index given by *YAC* target cells exposed to different antisera. Only such tests are included where both tests were performed simultaneously with the same sample of target cells. Correlation data: $r = 0{\cdot}61$, $P < 0{\cdot}001$, $Z = 0{\cdot}62$, $\sigma_z = 0{\cdot}12$, $P < 0{\cdot}001$

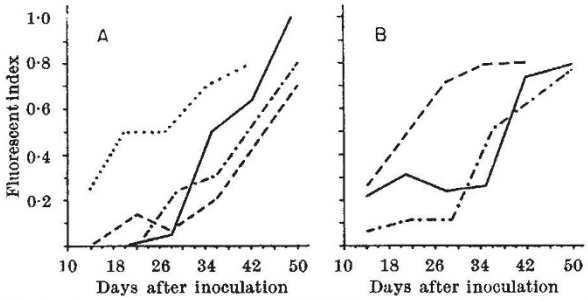

Fig. 2. Antibody response of $A \times C57Bl$ F_1 mice after the inoculation of 5×10^6 irradiated Moloney lymphoma cells, detected by the fluorescent method. *A*, ——, *YAC* sixth transfer; –·–·–·–, *YAC* eighteenth transfer; - - - - - -, *YAC* twenty-first transfer; . . . . . , *YDAB* first transfer—Target cells *YLD*. *B*, ——, *YAA* sixth transfer; –·–·–·–, *YAA* twenty-first transfer; - - - - -, *YHA* eighth transfer—Target cells *YAA*

presence of complement, and by the indirect fluorescent antibody reaction.

The detection, identification and quantitative titration of mouse leukæmia viruses have been largely dependent on biological induction tests. The difficulties encountered with the leukæmia viruses contrast sharply against the relative ease of virus assay in systems where suitable *in vitro* techniques are available, such as, for example, in the case of polyoma. Since humoral antibodies have been demonstrated in the Moloney system by the methods mentioned here, it should be possible to develop a mouse antibody production (MAP) test for virus assay. The usefulness of such tests has been demonstrated, for example, in the polyoma system[2], and the sensitivity was comparable with the plaque assay[3]. So long as the formation of the antibodies reacting with the target cells in the cytotoxic or the indirect fluorescent antibody test is induced by the virus, it is immaterial whether they are antiviral in nature or directed against virus-induced new cellular antigens, a question that has not been clarified for this system so far[1].

The work recorded here represents an attempt to examine the feasibility of working out a MAP test of this type for the assay of the Moloney lymphoma agent.

Sera of mice inoculated with materials to be assayed for their ability to induce specific antibody formation were tested each week by the indirect fluorescent antibody method and, in a number of cases, by the cytotoxic technique as well[1]. Moloney lymphomas were used as target cells; *YAC* of strain *A* origin most frequently (4th–20th passage in ascites form), *YAA* of strain *A* origin, *YDAB* of $A \times DBA$ F_1 origin, *YHA* of C_3H origin, and *YLD* of *C*57 leaden origin in a number of cases. The proportion of target cells killed in the cytotoxic test or stained in the indirect fluorescent test was expressed as a quantitative index in relation to corresponding controls incubated with control serum, as described in a previous publication[1]. Cytotoxic indexes $\geqq 0{\cdot}2$ and fluorescent indexes $\geqq 0{\cdot}3$ were regarded as positive. When the two types of tests were performed under standardized conditions, the cytotoxic and the fluorescent index increased as a function of time after the inoculation of virus-containing material. The scatter diagram in Fig. 1 shows the correlation between the cytotoxic and the fluorescent index obtained by exposing the same target cells to the same immune serum and indicates that the fluorescent method was more sensitive. A similar relationship was found when the titres given by the two methods were compared. For example, one serum that gave a cytotoxic titre of 1 : 20 against *YAC* had a 1 : 320 titre in the fluorescent test. Furthermore, while all Moloney lymphomas tested were suitable targets in the fluorescent test, some were not sensitive to the cytotoxic effect. The sensitivity of a given leukæmia varied only slightly between different cell pools in the fluorescent test, while the cytotoxic reaction was more variable. Critical comparisons between different sera of a given experimental series were always performed on the same cell suspension and at the same time. Apart from this relatively minor, uncontrollable variation, repeat tests with a given set of sera, a given target cell type, and a standard fluorescent serum or complement, resp., gave reproducible overall results in the fluorescent and the cytotoxic test as well.

Antibody response. The antibody response following the inoculation of a standard dose of X-irradiated Moloney lymphoma cells was determined for different lymphoma lines. The cells received 6,000 r. *in vitro* in order to prevent their proliferation *in vivo* which would bias the quantitation of the inoculum. Some typical experiments are shown in Fig. 2. In each group, 3 $A \times C57Bl$ F_1 male mice were inoculated with 5×10^6 irradiated cells. At different intervals, serum was collected and pooled from all three mice in each group and tested against Moloney target cells. Fig. 2*A* shows the antibody response by the fluorescence test after the inoculation of *YAC* cells from different passages and *YDAB* cells of the first passage, while Fig. 2*B* represents the reaction induced by *YHA* and *YAA* cells respectively. It appears that the time-period preceding the appearance of detectable antibody showed certain differences between the different lymphoma lines. Potent antibodies appeared earlier after the inoculation of *YHA* and *YDAB* (20–28 days) than after different transfer generations of *YAA* and *YAC* which were quite similar to each other, with antibodies appearing after

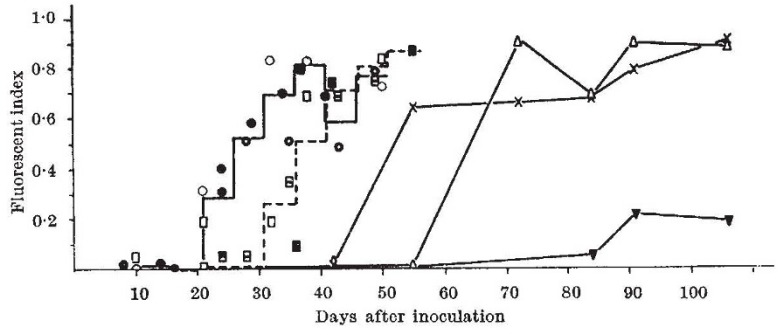

Fig. 3. Antibody response of $C3H \times C57Bl$ F_1 mice after the inoculation of virus containing homogenate, as detected by immunofluorescence. In four different experiments 0·1 ml. of 10^{-1} or 10^{-2} diluted homogenate was inoculated on day 0. In exps. 2, 3, 4 the same virus preparation was used (of $A \times DBA$ F_1 origin). In exp. 1 a different virus preparation was used of *C*57 leaden origin. The mean value of the fluorescent indexes for a given virus dilution was calculated for 5 days' intervals for dilutions 10^{-1} and 10^{-2}. In addition, dilutions 10^{-3}, 10^{-4} and 10^{-5} have been included in exp. 1. These are shown in the right. Symbols: circles, 10^{-1}; quadrangles, 10^{-2}; crosses, 10^{-3}; open triangles, 10^{-4}; closed triangles, 10^{-5}. Different shadings represent different experiments

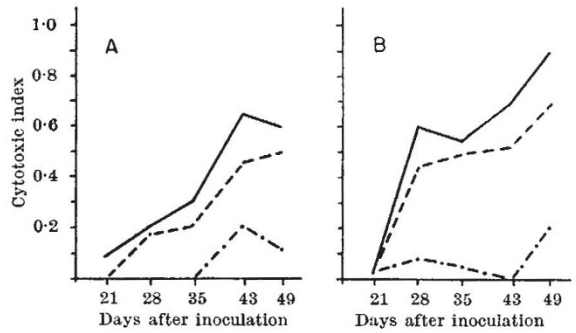

Fig. 4. Antibody response of groups of 3 *C3H* × *C57Bl* F_1 females, inoculated on day 0 with a standard virus containing homogenate (dilution: 10^{-1}). The serum pools were tested with *YAC* target cells. ——, no serum admixture; - - - - -, admixture of serum of untreated *C57Bl* × *DBA/2* F_1 mice; -.-.-.-, admixture of serum of *A* × *C57Bl* F_1 mice immunized with *YAA* Moloney lymphoma cells. *A*, cytotoxicity of serum pools in the presence of complement. *B*, fluorescent staining

35–42 days. A fifth lymphoma, *YLD*, not shown in the figures, behaved similarly to *YAC* and *YAA*. In order to examine the persistence of antibodies, mice inoculated with the same standard dose of irradiated cells from the five different lymphomas were also tested after 103–123 days. A high fluorescent index (range: 0·57–0·85) was found to persist in each case.

The maintenance of a high antibody-level during long periods of time after the inoculation of a single standard dose of irradiated, non-reproductive cells can be best interpreted by assuming that virus release and subsequent virus multiplication are responsible for the induction and persistence of antibodies. On this interpretation, all five tumour lines tested release virus, including those that have been carried through 18–22 passages, and two lines (*YHA* and *YDAB*) release larger quantities than the rest.

If the foregoing postulate is correct, inactivation of the virus should prevent antibody formation. This was tested by inoculating 5 × 10^6 cells of the *YHA* and *YLD* lines after they had been exposed to hydroxylamine[4] in order to inactivate the virus. The cell suspensions were mixed with equal volumes of 4 M hydroxylamine (*p*H 7) and incubated at 22° C for 15 min. The material was diluted 1 : 5 with phosphate buffered saline and dialysed against saline for 24 h. After inoculation, serum was collected after 7, 14, 30 and 75 days. There was no measurable antibody response.

Whether the antibodies detected are induced directly by the multiplying virus particles released by the tumour cells or by new cellular antigen(s) induced by the virus infection in the host cells cannot be decided on the basis of the present evidence.

The antibody response induced by different concentrations of the same virus preparation, preserved in the frozen state, was compared by the fluorescence test. Lymph nodes, spleen and liver of an *A* × *DBA* F_1 mouse with a primary Moloney lymphoma were homogenized in a glass homogenizer in 10 vol. of phosphate buffer and centrifuged for 20 min at 2,300*g*. The supernatant was centrifuged at 10,000*g* for 20 min and 0·1 vol. of the final supernatant were inoculated in 10^{-1} and 10^{-2} dilutions to each of 3 *C57* × *C3H* F_1 females. Three experiments were performed with the same virus pool. In a fourth experiment another homogenate, prepared from a primary lymphoma, induced in a *C57* leaden mouse, was inoculated in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. The pooled sera of the three mice in each experimental group were tested against Moloney target cells by the fluorescent method at different times after inoculation. The serum samples of each experimental series were tested on two or three different occasions against different samples of target cells. The mean fluorescent index was calculated for each dilution and each point of time. After the inoculation of 0·1 ml. 1 : 10 diluted virus, antibodies were detectable after 3 weeks (Fig. 3). A ten-fold dilution of the inoculated virus delayed the response by approximately 10 days.

Neutralization tests. Experiments were also made in order to detect virus neutralizing antibodies in the serum of resistant mice by testing the effect of serum on the immunogenic ability of virus-containing homogenates. A standard homogenate was diluted 10 and 100 times and 0·1-ml. vol. were inoculated to groups of 3 *C3H* × *C57Bl* F_1 females either alone, or after the admixture of 0·05-ml. serum. The serum has been derived from untreated *C57Bl* × *DBA/2* F_1 mice or from a pool collected from 16 *A* × *C57Bl* F_1 mice immunized 6–10 times with 10^5 or 10^6 viable Moloney lymphoma cells of strain *A* origin (lymphoma *YAA*). Specific antibodies against Moloney lymphoma cells could be demonstrated in this antiserum pool by the cytotoxic and the fluorescent test as well. The virus–serum mixtures and the untreated homogenates were incubated at room temperature for 30 min before inoculation. Antibody formation is shown in Figs. 4*A* and 4*B*. The immune serum neutralized the immunogenicity of the virus-containing homogenates. Comparison with the immunogenicity of different dilutions of the same standard homogenate indicates at least a hundred-fold neutralization. Analogous findings were obtained after the inoculation of the higher virus dilution. After the admixture of immune serum there was complete inhibition of antibody formation during the entire observation period of 66 days. The virus-neutralizing ability of anti-Moloney sera was also evident when oncogenic activity was used as test criterion. In this experiment new-born *A* × *DBA/2* F_1 mice were inoculated with 0·05-ml. virus containing homogenate each, mixed with 0·025-ml. serum. The results are shown in Table 1 indicating a clear-cut neutralizing effect.

Table 1. Leukæmia induction in *A* × *DBA* F_1 mice inoculated as new-borns (< 24 h) with virus preparation treated with serum from mice inoculated with Moloney leukæmia cells. Observation period 9 months

Dilution of virus	Virus preparation mixed with Control serum Incidence*	Latency period (days)	Immune serum Incidence*	Latency period (days)
10^{-1}	2/7	155, 192	1/23	226
10^{-2}	8/23	136, 210 208, 214 214, 218 218, 234	0/22	
10^{-3}	3/17	207, 234 239	0/16	

* Pooled serum from 15 *A* × *DBA* F_1 mice which received 5–8 inoculations of *YAA* Moloney leukæmia cells.

A number of other sera were also tested in the same way for a possible neutralizing effect. Results obtained with the 1 : 100 dilution series are exemplified in Table 2. Only the sera of mice resistant against Moloney lymphoma cells were able to neutralize the immunogenic effect of the inoculum, while anti-Gross, anti-6*C3HED* and anti-polyoma sera had no detectable effect. This is in accor-

Table 2. Effect of different antisera on the immunogenicity of Moloney-virus containing homogenates, as tested by the fluorescent antibody method. Three *C3H* × *C57Bl* F_1 mice were inoculated with the serum-homogenate mixture in each group

	Donor of serum admixed to homogenate Genotype	Pretreatment	Fluorescent index 21	31	40 Days after inoculation
Exp. 1	*C57Bl* × leaden F_1	—	0·12	0·35	0·55
	C3H	3 inoculations of 10^6 Moloney lymphoma cells, line *YHA*	0·09	0·00	0·03
	C3H	4 inoculations of 10^5 Gross lymphoma cells, line *GHA*	0·00	0·35	0·41
	C3H × *C57Bl* F_1	11 inoculations of 10^5 6*C3HED* lymphoma cells	0·32	0·44	0·41
	C3H	Inoculation of polyoma virus (HI titre of serum 1 : 1,840)	0·29	0·62	0·73
Exp. 2			16	24	37 Days after inoculation
	A.CA	—	0	0·07	0·68
	A.CA	10 inoculations of liver, spleen, lymph nodes of *A* animals (hæmaggl. titre: 1 : 10^4)	0	0	0·41
	A	6 inoculations of liver, spleen, lymph nodes of *A.CA* animals (hæmaggl. titre: 1 : 64)	0	0·03	0·91

dance with the findings obtained with the same types of sera when tested directly against Moloney cells[1].

The virus homogenate derived from $A \times DBA/2\ F_1$ lymphoma was also treated with H-2 isoantiserum against the virus-donor cell genotype on the assumption that host cell components may be incorporated into the coat of the virus particle. The results for the 10^{-2} dilution series are exemplified in Table 2, Exp. 2. There was no detectable neutralizing effect.

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MECHANISM OF ENZYME CATALYSIS

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ALTHOUGH numerous detailed investigations of enzymatic systems have been carried out, a basic understanding of enzyme catalysis is still lacking. In particular, two fundamental questions are still unanswered: (1) Why are enzymes such efficient catalysts? (usually at least a factor of 10^6 better than the corresponding synthetic catalysts); (2) Why is a macromolecule required? The two questions, of course, are not unrelated and numerous speculations have been made. The present belief is that the macromolecular conformation is all-important, although very few specific suggestions have been put forth of a quantitative nature. The idea that conformation can control enzyme specificity is easy to understand qualitatively and was embodied long ago in the 'lock and key' hypothesis of Emil Fischer[1]. Several variations of the 'lock and key' idea are now in vogue. One school of thought believes that only a few side-chain groups are really important, and that by bringing these critical groups together in a stereospecific fashion, efficient catalysis ensues. A different variation on the same theme is embodied in the 'induced fit' theory put forth by Koshland[2], who suggests that the substrate induces changes in the protein conformation which aid in the substrate binding and catalysis. In this case the protein conformation changes in a dynamic manner throughout the course of the reaction. Eyring and Lumry[3] have suggested that the protein may serve as a 'rack' which distorts the critical substrate bonds thereby promoting catalysis. Unfortunately, none of these models can really be tested experimentally in a critical fashion. In a few cases (for example, chymotrypsin[4]) the available evidence suggests that the protein conformation does change during the course of the reaction; however, this fact is consistent with virtually all present proposals concerning the mechanism of enzyme catalysis.

The purpose of this article is to put forward some general ideas which might be of possible relevance in understanding enzyme catalysis.

In order to put the ideas presented in some sort of framework, I shall use the language of transition state theory and recall that the overall standard free energy change for a given reaction is unaffected by the presence of catalysis. Obviously, this same statement is also valid concerning the standard enthalpy and entropy changes for a particular reaction. The catalyst, however, does significantly lower the standard free energy of activation since this quantity directly reflects the efficiency of the catalytic process. Let us now consider how the enthalpy and entropy of activation may be affected by an enzyme. Both factors are undoubtedly influenced by the catalytic process although present evidence suggests that entropic effects may predominate.

Enthalpy effects. Enzymes can bring about a lowering of the activation energy in several ways. One of the most probable methods is a 'compensation' effect. By forming intermediate compounds the breaking of a bond can be facilitated; a possible way of accomplishing this is by simultaneously breaking and forming bonds. Several clear-cut examples of this phenomenon as found in elementary gas phase reactions are assembled in Table 1. For example, the activation energy for decomposition of H_2 by an inert body is greater than 100 kcal/mole, while that for the reaction $H_2 + I_2 \rightarrow 2HI$ is about 40·7 kcal/mole. Thus the activation energy for the second reaction is smaller even though an H_2 bond is still broken in the overall process. In general, the greater the energy involved in the bond formed, the more the activation energy is lowered. An analogous possibility for enzyme catalysis is that side-chain interactions such as H bonds, hydrophobic bonds, etc., are involved in the formation of 'compensating bonds'. A schematic representation of this idea is shown in Fig. 1. Such effects could involve co-operative phenomena so that protein groups not directly at the active site could promote catalysis. Of course, we must ultimately pay for this by breaking the side-chain bonds to get the catalyst back in its original state. However, such processes have small activation energies so that overall the activation energy of the reaction would be lowered. For example, a hydrogen bond can be broken in about 10^{-9} sec[6], solvation structure can be altered in about this same time[7], and the helix–coil transition in polyglutamic acid occurs in less than 10^{-5} sec[8]. Another more obvious example of this type of compensation occurs when part of the substrate molecule forms a covalent intermediate with the enzyme (for example, chymotrypsin[9], phosphoglucomutase[10], etc.).

Table 1. Examples of Energy Compensation in Elementary Gas Phase Reactions*

Reaction	Bond broken	E_a(kcal/mole)
$H_2 \rightarrow 2H$	H–H	104·0
$H_2 + I_2 \rightarrow 2HI$	H–H	40·7
$H_2 + Cl \rightarrow HCl + H$	H–H	4·6–6·1
$H_2 + Br \rightarrow HBr + H$	H–H	17·6
$Cl_2 \rightarrow 2Cl$	Cl–Cl	58·0
$H + Cl_2 \rightarrow HCl + Cl$	Cl–Cl	2·4
$Br_2 \rightarrow 2Br$	Br–Br	92·0
$H + Br_2 \rightarrow HBr + Br$	Br–Br	0·9
$CH_4 \rightarrow CH_3 + H$	C–H	102·0
$CH_3 + C_2H_6 \rightarrow CH_4 + C_2H_5$	C–H	10·4
$H + C_2H_6 \rightarrow C_2H_5 + H_2$	C–H	6·4

* Compiled from ref. 5.

One of the most commonly accepted ideas concerning the role of the enzyme in catalysis is that it rigidly holds the substrates in place so that proton or electron transfer can occur readily. This is undoubtedly at least partially true and may be predominantly an entropic effect. How-

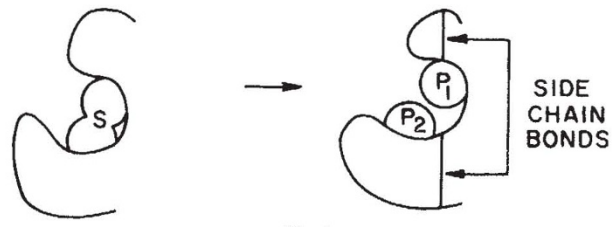

Fig. 1